Drought, disease or devil declines? Identifying the cause of decline of the eastern quoll, *Dasyurus viverrinus*

Implications for conservation and management

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"The extinction problem has little to do with the death rattle of the final actor.
The curtain in the last act is but a punctuation mark – it is not interesting in itself.
What biologists want to know about is the process of decline in range and numbers"

Michael E. Soulé (1983)

Statements by the author

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The research associated with this thesis abides by the *Australian code of practice for the care and use of animals for scientific purposes* (2004) and the rulings of the Animal Ethics Committee of the University. The research presented in this thesis was carried out under University of Tasmania Animal Ethics Approvals A11017 and A11655, and Tasmanian Department of Primary Industries, Parks, Water and Environment scientific permits FA10042, FA10116, FA11050, FA11208, FA11295, FA12048, FA12143, FA13060 and FA13909.

Bronwyn A. Fancourt
13 April 2015

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- **Stewart Nicol** and **Menna Jones** contributed to ideas and edited manuscripts for all chapters except Appendix A.
- **Clare Hawkins** contributed to ideas and edited all manuscripts except Chapter 4 and Appendix A.
- **Elissa Cameron** contributed to ideas for Chapter 5 and edited the manuscripts for Chapters 3 and 5.
- **Robert Jackson** contributed to data collection for Chapter 4 and edited the manuscript.
- **Brooke Bateman** contributed to the study design and data analysis for Chapter 2 and edited the manuscript.
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Abstract

Diagnosing the cause of a species' decline is one of the most challenging tasks faced by conservation practitioners. A decline in abundance may simply be part of a natural population fluctuation from which the species will recover without management intervention, or it may indicate a more concerning trajectory towards extinction. Different threats and mechanisms can operate at different temporal and spatial scales, in succession or simultaneously. Often, several threats act together to produce synergistic effects that are greater than the sum of the contributions of each threatening process in isolation. Effective conservation strategies require an understanding of the factors that threaten a species and how those factors interact.

The eastern quoll (*Dasyurus viverrinus*) is a medium-sized carnivorous marsupial that is extinct on the Australian mainland and survives only in Tasmania. The species has declined by more than 50% in the 10 years to 2009, with no sign of recovery. The reasons for this precipitous decline are not currently understood. Population eruptions and declines have been anecdotally reported in eastern quolls since the 1800s, suggesting that the species may be sensitive to short-term variations in weather. Additionally, a recent study suggested that the decline of the Tasmanian devil (*Sarcophilus harrisii*) due to the spread of the Devil Facial Tumour Disease (DFTD) may have released feral cats (*Felis catus*) from competitive suppression. A subsequent increase in cat sightings might be linked to eastern quoll declines, possibly through mechanisms such as increased predation, competition or exposure to toxoplasmosis, the disease caused by the cat-borne coccidian parasite *Toxoplasma gondii*.

The aim of this study was to investigate a number of candidate causal agents to determine which factors have contributed to the recent decline of the eastern quoll in Tasmania.

To test if shifting weather patterns explain the recent eastern quoll decline, I developed a temporally explicit species distribution model using short-term weather variables matched to quoll occurrence records between 1950 and 2009. I used the model to reconstruct variation through time in the distribution of climatically suitable range for the species. Abundance of quolls, indexed by transect counts, was positively related to the modelled area of suitable habitat between 1990 and 2004. A sharp decline in the abundance index from 2001 to 2003 coincided with a sustained period of unsuitable weather over much of the species' distribution. Since 2004, abundance has not recovered despite a return of suitable conditions, and abundance and area of suitable habitat have been uncorrelated. I suggest that fluctuations in weather account for the species' recent decline, but that other unrelated factors have suppressed recovery.

I tested the effects of *T. gondii* infection on eastern quolls by regularly screening quoll populations at four sites for the seroprevalence of *T. gondii*-specific IgG antibodies. Seroprevalence was five times higher at sites with declined quoll populations, and there was a negative association between seroprevalence and quoll abundance. However, *T. gondii* infection did not reduce quoll survival or reproduction. Despite a high susceptibility to *T. gondii* infection, eastern quoll populations did not appear to be limited by the parasite or its resultant disease. Higher seroprevalence in quolls was not attributable to higher *T. gondii* prevalence in feral cats, but rather signalled greater exposure to feral cats at sites where eastern quolls had declined. I therefore suggest that increased predation, competition or exclusion by feral cats may be contributing to quoll declines or inhibiting their recovery from low abundance.

I also investigated the influences of top-down effects on abundance and activity patterns among devils, feral cats and eastern quolls. Throughout the eastern quoll's range, I carried out a combination of longitudinal trapping and camera surveys. I found no evidence of a negative relationship between devil and cat abundance, and also no evidence of higher cat abundance in areas where devil populations had declined the longest. While cats did not appear to avoid devils spatially, there was some evidence suggestive of temporal avoidance. Cat and devil activity showed marked separation, with reduced separation observed in areas where devils had declined the longest. Cats and quolls used the same areas, and there was no evidence that cat and quoll abundance were negatively related. However, temporal overlap in cat and quoll activity was higher in summer than in winter, implying a high risk of predation for juvenile quolls (which emerge in summer). I suggest that predation of juvenile quolls by cats may be inhibiting low density-quoll populations from recovering their former abundance following weather-induced decline, but that this is independent of devil decline.

This study demonstrates how multiple threatening processes can interact to bring about the decline of a common species and inhibit its recovery. Confounding variables and mechanisms can operate at different temporal and spatial scales, such that contemporary agents of decline may be unrelated or disconnected from the original cause of decline. Residual small populations are inherently more susceptible to demographic, environmental and genetic stochasticity and are unlikely to recover without management intervention.

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List of abbreviations

AI	Abundance index
ANOVA	Analysis of variance
AUC	Area under the receiver operating curve
AWAP	Australian Water Availability Project
BACI	Before-after-control-impact
BCI	Body condition index
BI	Bruny Island (north)
CI	Confidence interval
CM	Cradle Mountain
CR	Cradoc
CWR	Critical weight range
DAT	Direct agglutination test
DPIPWE	Department of Primary Industries, Parks, Water and Environment, Tasmania
DFTD	Devil Facial Tumour Disease
ELISA	Enzyme-linked immunosorbent assay
FGA	First generation anticoagulant
GLMM	Generalised linear mixed model
IFAT	Indirect fluorescent antibody test
IHA	Indirect haemagglutination assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
JU	Judbury
KM	Kaplan Meier
LAT	Latex agglutination test
MAT	Modified agglutination test
NS	Not specified
OR	Odds ratio
PY	Pouch young
RN	Royle Nichols
SA	South Australia
SBI	Bruny Island (south)
SDM	Species distribution model
s.d.	Standard deviation
s.e.	Standard error
SGA	Second generation anticoagulant
TL	Testicular length
TV	Testicular volume
TW	Testicular width
WA	Western Australia

Chapter 1
General Introduction

Black eastern quoll at Cradle Mountain, Tasmania (Photo: Alison Fancourt).

1.1 Australia's disappearing mammalian fauna

Australian ecosystems have been profoundly altered since European settlement in 1788, leading to changes in the richness, composition, abundance and distribution of Australia's mammalian fauna (Burbidge *et al.* 2008; Woinarski *et al.* 2014). The introduction of non-native grazers and predators, widespread land clearance and modification, expansive agriculture, hunting, poisoning, persecution and changed fire regimes have all contributed to an increasingly depauperate native fauna and a shift to invasive-dominated systems. In just over 200 years, 28 (10.3%) of Australia's 271 endemic mammal species have become extinct and a further 55 (20.3%) species are considered threatened. This is a rate of decline unparalleled on any other continent in recent history (Baillie *et al.* 2004; McKenzie *et al.* 2007; Woinarski *et al.* 2014). However, this deterioration has not occurred evenly throughout Australia's fauna. Terrestrial mammals have been particularly susceptible to extinctions and declines in both range and abundance. 'Critical Weight Range' (CWR) species (non-flying mammals with a mean adult body mass between 35 g and 5500 g) have suffered at disproportionately high rates (Burbidge and McKenzie 1989; Johnson 2006; McKenzie *et al.* 2007; Johnson and Isaac 2009; Woinarski *et al.* 2014).

1.2 Tasmania: an island refuge for Australia's mammals

With the notable exception of the thylacine (*Thylacinus cynocephalus*) (McKnight 2008b), the island state of Tasmania (68 400 km^2) has remained largely unaffected by the mammalian extinctions and declines that have devastated the Australian mainland (Short and Smith 1994; Woinarski *et al.* 2014). Five species that were once widespread on the mainland now survive only in Tasmania, while several other species that have suffered dramatic reductions in their mainland range and abundance still persist in comparatively higher densities in this island refuge (Burbidge 1999; Woinarski *et al.* 2014). The historic absence of the European red fox (*Vulpes vulpes*) has likely facilitated the persistence, diversity and abundance of CWR species on the island (Johnson 2006). However, Tasmania's relatively intact guild of large marsupial carnivore species may also have contributed to marsupial persistence. The Tasmanian devil (*Sarcophilus harrisii*), spotted-tailed quoll (*Dasyurus maculatus*) and eastern quoll (*Dasyurus viverrinus*) are thought to

regulate ecosystem function through suppressing the impacts of introduced species such as the black rat (*Rattus rattus*), European rabbit (*Oryctolagus cuniculus*) and feral cat (*Felis catus*) (Wood Jones 1923; Jones *et al.* 2007; Peacock and Abbott 2013).

While Tasmania remains a stronghold for marsupial diversity in Australia, ecosystem dynamics are changing and new threats are emerging. The fox was introduced to the island c. 1999-2001 (Saunders *et al.* 2006; Sarre *et al.* 2012), and the largest mammalian carnivore, the Tasmanian devil, has been in rapid and steep decline since 1996 due to the spread of the fatal Devil Facial Tumour Disease (DFTD) (Hawkins *et al.* 2006). There is concern that the ongoing loss of devils may release invasive mesopredators such as feral cats and cause changes in populations of prey species, triggering unprecedented trophic cascades that could threaten a range of animal and plant species (Jones *et al.* 2007).

1.3 The decline of the eastern quoll

The eastern quoll is a medium-sized carnivorous marsupial that was once widespread throughout south-eastern Australia, but now survives only in Tasmania. Mainland populations declined rapidly around the late 1800s and early 1900s (Wood Jones 1923; Peacock and Abbott 2014). The species persisted in relatively low densities within a greatly reduced range, until the last confirmed sighting in Sydney in 1963 (Dickman *et al.* 2001). In contrast to its mainland extirpation, the eastern quoll continued to thrive in Tasmania (Green 1967) where it was considered stable and secure (McKnight 2008a). However, the species has recently undergone rapid and severe population decline in Tasmania (Figure 1.1; Fancourt *et al.* 2013). A combination of trapping and spotlight surveys indicated statewide declines of more than 50% in the 10 years to 2009 with no sign of recovery (Fancourt *et al.* 2013). The reasons for this precipitous and ongoing decline are not currently understood.

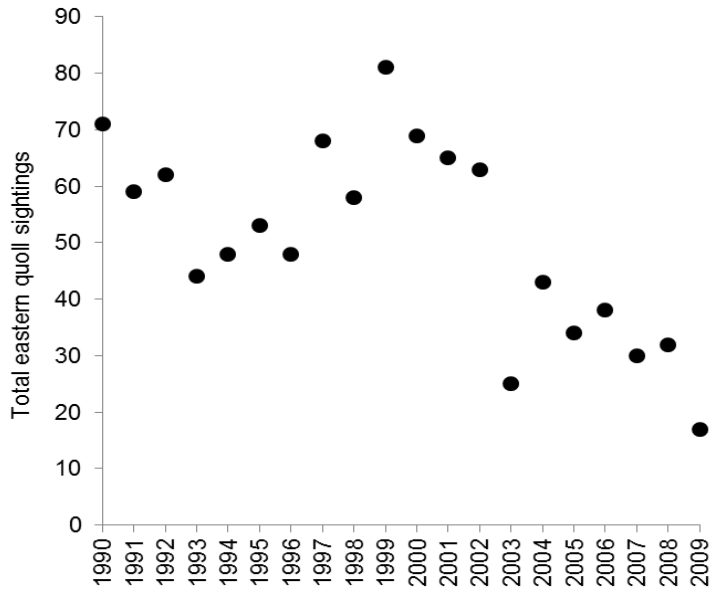


Figure 1.1. Total number of eastern quoll sightings in Tasmania, recorded in annual spotlight surveys across transects (*n* = 147) surveyed every year between 1990 and 2009 inclusive (reproduced from Fancourt *et al.* 2013: p. 199). Transects cover most regions across mainland Tasmania, excluding the far west and south-west of the state.

1.4 Diagnosing the cause of decline

Diagnosing the cause of a species' decline is one of the most challenging tasks faced by conservation practitioners (Caughley 1994). A population decline may result from a contraction in a species' range, or a decline in abundance within an existing range (Rodríguez 2002). For some species, a decline in abundance may simply be part of a natural population fluctuation from which the species will recover without management intervention (Krebs *et al.* 2001). Alternatively, it may indicate a more concerning trajectory towards extinction (O'Grady *et al.* 2004). Threatening processes can act alone or in combination. Multiple threats often act together to produce synergistic effects that are greater than the sum of the contribution of each threatening process in isolation (Brook *et al.* 2008). Accordingly, before appropriate conservation strategies can be developed, managers need to understand the factors that determine and limit the species' distribution and abundance.

To identify the responsible agent(s) of a species' decline, Caughley (1994) proposed a series of four steps (Figure 1.2):

1. Gain an understanding of the species' natural history, ecology, context and status;
2. Based on the knowledge gleaned in step 1, list all the conceivable agents of decline;
3. Measure and contrast the agents where the species is now, and where the species used to be, to identify putative causal agents of decline; and
4. Test the hypotheses produced from step 3 to confirm agents are causal and not merely associated with the decline.

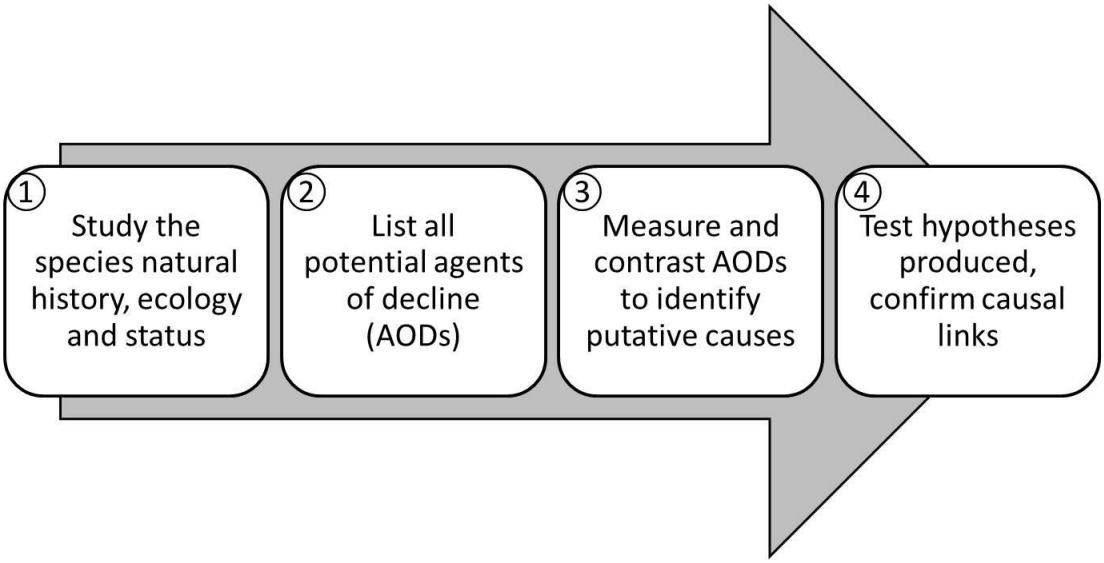


Figure 1.2. Graphical representation of Caughley's (1994) framework for diagnosing the cause of a species' decline.

While this diagnostic framework provides a solid scientific foundation to simplify many complex and difficult investigations, its application and the usefulness of any insights gained may be limited. For example, inherent in step three is the necessity that the species' decline is still ongoing, or has not yet reached a state where all populations have declined or become locally extinct, thereby facilitating comparison between populations that have declined and those that have not. Confounding variables and mechanisms can operate at different temporal and spatial scales, both in succession and simultaneously (Elliott and Brook 2007). This is often the case for a species undergoing decline, where the

final step in the extinction vortex may be unrelated or disconnected from the original cause of decline (Brook *et al.* 2008). For a species approaching extinction, we are unable to go back in time to measure the agents that operated at various stages of the decline. In such cases, we are restricted to measuring those factors currently operating on remaining populations (which may or may not be related to factors operating earlier in the decline), and inferring other mechanisms from different lines of evidence (Hillborn and Mangel 1997; Elliott and Brook 2007).

Another limitation of Caughley's (1994) approach is that it may encourage focus on a single working hypothesis at the expense of alternative and interacting hypotheses. Such an approach may still be appropriate if a simple explanation will suffice, or where multiple factors occur in succession to bring about a species decline (Figure 1.3(a)) (Elliott and Brook 2007). For example, parasitised animals may subsequently be more vulnerable to predation (Barber *et al.* 2000; Berdoy *et al.* 2000). If multiple factors operate in parallel (Figure 1.3(b)), then the investigation should focus instead on the relative importance of each factor, and how they may interact (Elliott and Brook 2007).

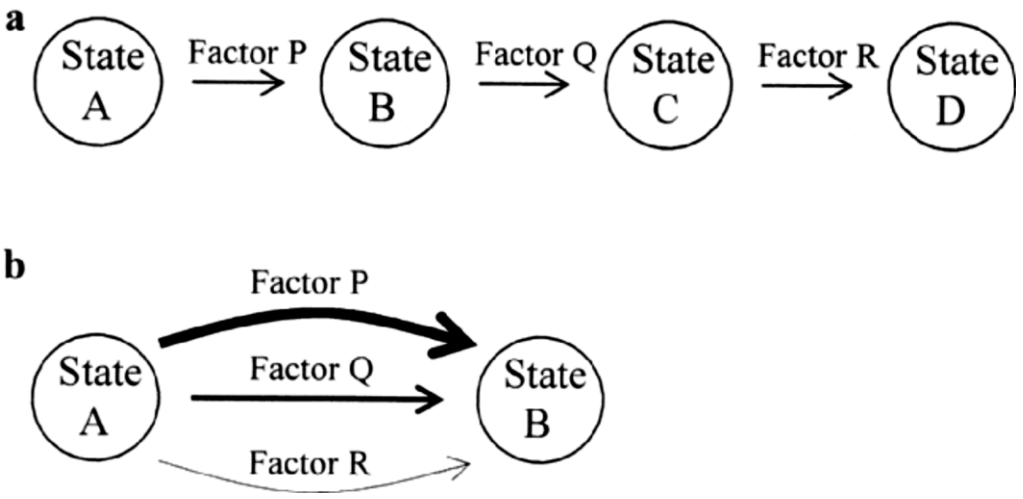


Figure 1.3. Conceptual models of how multiple factors can lead to a state transition, both (a) in series, where two or more factors occur sequentially; and (b) in parallel, where the relative strength of simultaneous factors is indicated by the line thickness (reproduced from Elliott and Brook 2007: p. 610).

In developing the method of multiple working hypotheses, Chamberlin (1890) asserted that scientists should begin the task of explaining an observation by forming all reasonable hypotheses of its cause. While this approach is analogous to step two in Caughley's (1994) framework, Chamberlin's (1890) approach is potentially more comprehensive in that it recognises the possibility that more than one hypothesis may be simultaneously true. For example, if an agent is found to be causal at step four in Caughley's (1994) framework, we may prematurely conclude that the decline is attributable to this single factor alone. However, in doing so, we fail to recognise that it could be but one factor, and possibly only a minor factor, in the accomplishment of the final result (Chamberlin 1890).

In this study, I have adopted Caughley's (1994) diagnostic framework as a basis for commencing an investigation into the cause(s) of decline of the eastern quoll. I have taken this approach for two reasons. First, the decline of the eastern quoll is still in progress, and some populations have not (yet) declined. This means that it should be possible to directly study the mechanisms of decline by comparing declining and non-declining populations. Second, by comparing declining with non-declining populations or comparing populations in different stages of decline, I can identify factors associated with the decline and study their effects. If multiple factors are found to be associated, knowledge of their effects will allow me to hypothesise as to whether they may have acted sequentially or simultaneously, and to subsequently determine the most appropriate approach for testing multiple working hypotheses in step four.

Here, I address the first two steps in the process: outlining the eastern quolls' natural history and ecology, and compiling a list of the potential agents of decline based on available circumstantial and preliminary evidence. I address step three for the most likely candidate agents of decline in Chapters 2 to 5. In Chapter 6, I synthesise the evidence for each of the candidate agents investigated in context, and provide a testable hypothesis as to the causes of decline of the eastern quoll. This hypothesis should form the basis of future research to address step four.

1.4.1 Step 1: Natural history, ecology and status of the eastern quoll

The eastern quoll is a medium-sized sexually dimorphic marsupial carnivore with a mean adult body mass of 1250 g (900–2000 g) for males and 850 g (700–1100 g) for females (Godsell 1983; Jones and Rose 2001). The pelage is either tan or black in colour, with white spots across the entire body, except the tail (Figure 1.4). Females are seasonally polyoestrous, while sexual activity in males is typically restricted to between April and June each year (Godsell 1983; Fletcher 1985). Individuals of both sexes are sexually mature in their first year, and females concentrate reproductive effort in their first two breeding years (Godsell 1983; Bryant 1988). Births are highly synchronous in June-July each year. The mother carries a maximum of six young in the pouch for around 8-9 weeks, then deposits them in a den until they are fully weaned at around 20-30 weeks of age, with duration dependent on litter size (Godsell 1983; Merchant *et al.* 1984). This highly synchronous breeding typically results in a 3- to 4-fold increase in population abundance around November and December each year when newly weaned quolls first emerge from their natal dens as independent juveniles. Population abundance typically remains high until after the May-June mating season, after which populations usually return to pre-weaning abundance (Godsell 1983). Local activity of males increases over the May-June mating season each year. The more mobile males cover a mean home range of around 44 ha compared to 35 ha for females (Godsell 1982; 1983; Bryant 1986), although larger home ranges have been observed in sub-alpine areas (M. Jones, unpubl. data). Annual mortality appears high (Godsell 1983), although the causes remain unclear and speculative (Dickman *et al.* 2001). Maximum life expectancy is around 3-4 years in the wild (Godsell 1983).

The eastern quoll is widespread throughout most of Tasmania. It occurs primarily in the drier agricultural regions in the eastern half of the island, although it is infrequently observed in low densities in open habitat throughout the wetter west of the island (Jones and Rose 1996). It is commonly associated with forest-pasture interfaces that provide open grasslands for foraging at night, adjoining natural forest habitat where quolls den in hollow logs, under rocks and in underground burrows during the day (Godsell 1983). It also occurs in sub-alpine buttongrass (*Gymnoschoerus sphaerocephalus*) moorlands, sedgelands and a mix of wet and dry sclerophyll forest, but is notably absent from large

(a)

(b)

Figure 1.4. The two pelage colours of the eastern quoll, (a) tan form (sometimes referred to as fawn, beige, light brown, grey or olive), and (b) black form (*Photos: Bronwyn Fancourt*).

tracts of rainforest (Rounsevell *et al.* 1991; Taylor and Comfort 1993; Fancourt *et al.* 2013). The diet consists mostly of invertebrates, although birds, small mammals, reptiles, fruit and carrion are also eaten depending on season and location (Blackhall 1980; Godsell 1983; Jones and Barmuta 1998).

The ecological interactions between eastern quolls and their potential predators and competitors are not well understood. Eastern quoll remains have been found in roost and nest sites of the masked owl (*Tyto novaehollandiae*) (Mooney 1993), and cats are known to kill eastern quolls (Peacock and Abbott 2014; B. Fancourt, unpubl. data), although the frequency and impacts of predation on eastern quoll populations are not currently known. Tasmanian devils are known to scavenge dead quolls (Jones 2000) and display competitive aggression towards them when feeding around carcasses (Jones 1998), however it is unclear whether devils or spotted-tailed quolls hunt or kill live eastern quolls. Adult male eastern quolls display anti-predator behaviours to vocalisations of devils and masked owls, and juvenile males additionally respond to feral cats, indicating that quolls may perceive these Tasmanian predators as a threat (Jones *et al.* 2004). Male eastern quolls were found to exhibit some dietary overlap with smaller spotted-tailed quolls at Cradle Mountain at certain times of year (Jones and Barmuta 1998). Some dietary overlap between eastern quolls and feral cats may be inferred from species-specific dietary studies (e.g. Blackhall 1980; Godsell 1983; Jones and Barmuta 1998; Lazenby 2012). However, such Tasmanian studies are limited both spatially and temporally, and no studies have investigated the diets of sympatric cats and quolls in Tasmania. Therefore, the extent to which eastern quolls may compete with feral cats for resources is largely unknown.

1.4.2 Step 2: Potential agents of decline

In compiling a list of potential agents of decline, I have drawn on two main lines of evidence. First, I examine the factors implicated in the eastern quoll's demise on the Australian mainland. Second, I consider a range of factors that have occurred or changed in Tasmania over recent decades that broadly correlate temporally with the period of eastern quoll decline. Each of these factors is discussed below.

1.4.2.1 Climatic variables

Population eruptions and declines have been anecdotally reported in eastern quolls since the 1800s, both in Tasmania and on the mainland (Peacock and Abbott 2014). These observations lend support to the hypothesis that marked fluctuations may simply be part of the species' natural history. However the mechanisms driving these fluctuations are not understood. Unfavourable climatic conditions may contribute to population declines by exceeding a species' physiological tolerances (Root 1988; St. Clair and Gregory 1990), limiting food resources (Thomas *et al.* 1996) or disrupting reproduction and completion of life cycles (Woodward *et al.* 1990). Furthermore, climate change can exacerbate extrinsic threats such as disease (Pounds *et al.* 2006). Short-term weather fluctuations and extreme climatic events can result in sudden marked changes in a species' distribution and abundance (Parmesan *et al.* 2000; Whitfield *et al.* 2007).

Weather extremes are a candidate agent in the eastern quoll decline, and the mechanism of their role has great implications for conservation management. The recent decline in Tasmania coincides approximately with 'the millennium drought' (2001-2009), the longest uninterrupted series of years with below median rainfall in southeast Australia since at least 1900 (van Dijk *et al.* 2013). If weather extremes drive fluctuations in quoll abundance, the recent decline may be temporary and recovery could ensue without management intervention when weather conditions return to normal. Alternatively, the recent decline could represent a cumulative or permanent trajectory towards extinction (Ehrlich *et al.* 1980; Thomas *et al.* 1996). Therefore, as a first step, it is imperative that the nature of the decline be determined by investigating if the distribution and abundance of eastern quolls are sensitive to short-term variations in climatic variables (i.e. weather), and if shifting weather patterns can explain the recent decline.

1.4.2.2 Feral cats

Predation by feral cats is considered to be the most significant factor in Australia's recent mammalian extinctions, and is also regarded as the factor affecting the largest number of threatened and near threatened mammal taxa in Australia (Figure 1.5; Woinarski *et al.* 2014). Since the 1860s, there have been reports of domestic cats killing quolls (Peacock and Abbott 2014), indicating that feral cats are capable of killing adult and juvenile eastern quolls. However, most historic observations involve domestic cats, and the extent

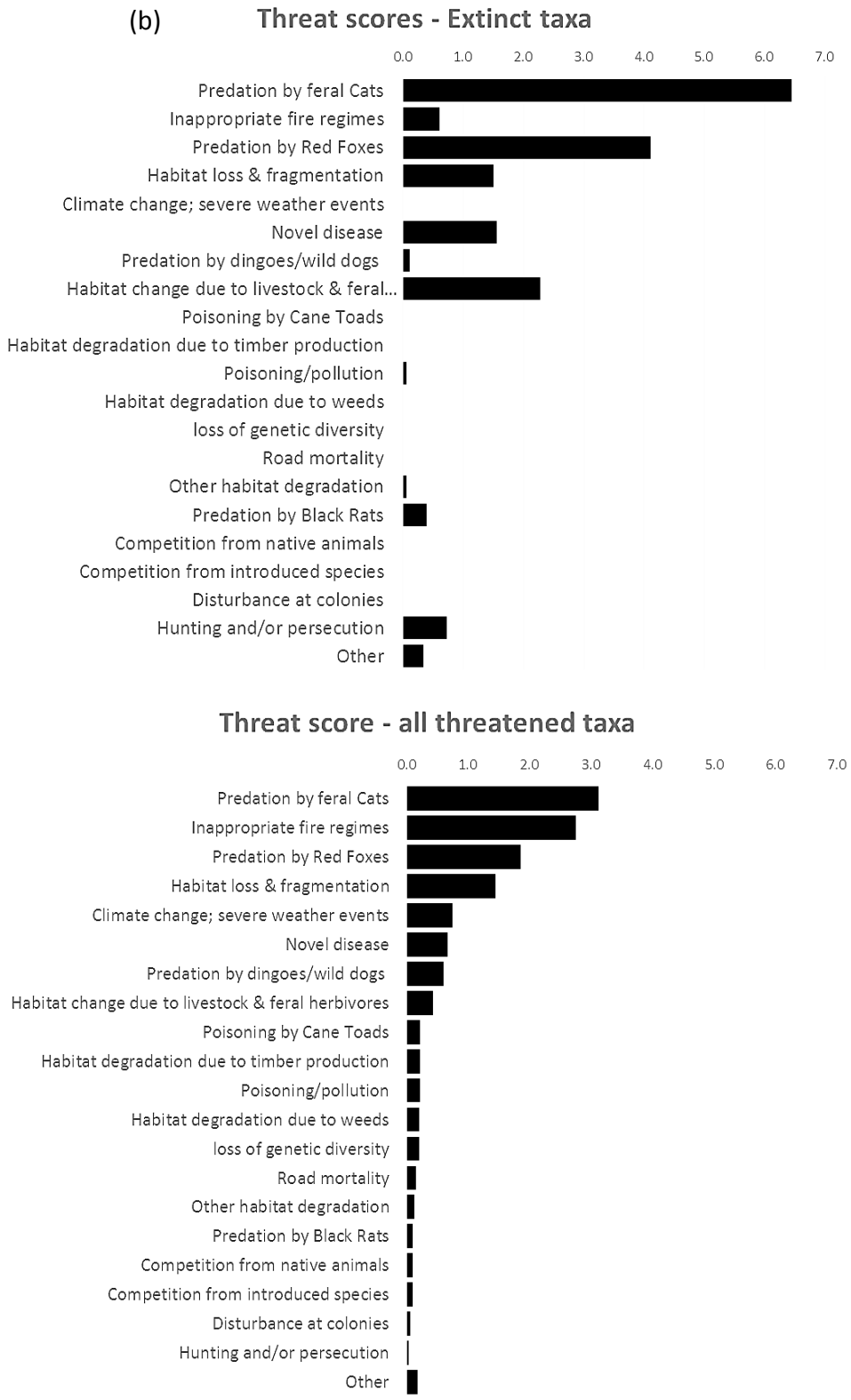


Figure 1.5. Mean threat scores for all extinct mammal taxa (a) and threatened terrestrial mammal taxa (b), from *The Action Plan for Australian Mammals 2012* (reproduced from Woinarski *et al.* 2014: p. 871). Threats were rated according to severity and extent by a number of experts for each species. Resulting threat scores were tallied across groups of taxa of different conservation status and averaged across the number of taxa considered.

to which feral cats may have contributed to historic quoll declines on the mainland is unknown. Domestic cats were first introduced to Tasmania in 1806 while the earliest records of feral cats are from the 1840s (Abbott 2008). Accordingly, cats and quolls have not only co-existed but thrived together in Tasmania for over 200 years, without any known significant negative effects on populations of either species. This suggests that feral cats are unlikely to have been a major contributor to the recent quoll decline. However, historical records and recent studies suggest that feral cats sometimes act in conjunction with a range of other variables such as alteration of habitat, fire, drought and disease to contribute to the decline of native taxa (Oakwood 2000; Burbidge and Manly 2002; Abbott 2006; McGregor *et al.* 2014). This lends support to the hypothesis that variables such as 'the millennium drought' (Tasmanian Planning Commission 2009; van Dijk *et al.* 2013) in combination with ongoing habitat changes (Forest Practices Authority 2012) may have been enough to unsettle the historic balance between these species in favour of cats, possibly contributing to the recent decline in eastern quolls.

Of particular note is the decline of the Tasmanian devil due to the spread of the fatal DFTD (Hawkins *et al.* 2006). As the largest terrestrial carnivore on the island, it has been hypothesised that devils historically suppressed feral cats, through aggressive encounters, competition and possibly predation (Jones *et al.* 2007). If this is the case, then the ongoing loss of devils may release feral cats, potentially allowing them to alter their spatial and temporal activity and possibly increase in abundance. While the interactions between devils, cats and eastern quolls are currently unknown, any increase in feral cat abundance or activity may exert additional pressure on smaller predators such as the eastern quoll, possibly through increased predation, exploitation or interference competition, or exposure to diseases such as toxoplasmosis. Accordingly, feral cats are a candidate causal factor in the recent quoll decline.

1.4.2.3 Disease

Numerous historical accounts refer to an unspecified disease that affected eastern quolls on the mainland commencing around the mid-1860s, with the number of accounts peaking between 1890 and 1910 (Peacock and Abbott 2014). In some areas, local quoll populations seemingly disappeared within a matter of weeks or months (Peacock and Abbott 2014), although some populations persisted in relatively low densities in a few

areas until the 1950s or 1960s (Lindsay 1962; Wakefield 1964; Seebeck 1984). Many have speculated as to the identity of the candidate pathogen or disease: mange, heavy ectoparasite burdens, bubonic plague, a distemper-like virus, and toxoplasmosis have all been suggested (Peacock and Abbott 2014).

There is some evidence that the mainland disease was not host-specific. While the exact causative agent(s) is unknown, several accounts refer to disease affecting a range of native animals at that time, including possums, phascogales, bettongs, wallabies, kangaroos and koalas (Lindsay 1962; Lunney and Leary 1988; Curson and McCracken 1989; Recher *et al.* 1993; Abbott 2006; Peacock and Abbott 2014).

There is no evidence for a pathogen that is not host-specific being involved in the eastern quoll's recent Tasmanian decline. In Tasmania, comparable declines in a range of marsupials have not been observed. The only confirmed disease-induced species decline during the period of quoll decline is that of the Tasmanian devil due to the spread of DFTD (Hawkins *et al.* 2006). While the close relatedness of eastern quolls to devils may imply a similar susceptibility, the cell line responsible for this infectious cancer is considered highly unlikely to grow in other species (McCallum and Jones 2006). To date, no cases of DFTD have been confirmed in any related species.

Toxoplasmosis, the disease caused by the pathogen *Toxoplasma gondii*, has been posited as the disease possibly responsible for the historic eastern quoll declines on the mainland (Shepherd and Mahood 1978; Cross 1990; Freeland 1993; Recher *et al.* 1993). *T. gondii* is an intracellular coccidian parasite with a worldwide distribution (Hill *et al.* 2005; Dubey 2010). Infection by *T. gondii* can result in overt clinical disease (Dubey and Frenkel 1972; Innes 1997; Dubey 2010), with fatalities observed in many wildlife species (Work *et al.* 2000; Szabo *et al.* 2004; Jokelainen and Nylund 2012; Howe *et al.* 2014). Some Australian marsupials are especially susceptible to toxoplasmosis (Obendorf and Munday 1983; Canfield *et al.* 1990; Innes 1997; Bettiol *et al.* 2000). In Australia, feral, stray and domestic cats are the only definitive host that can excrete the environmentally persistent *T. gondii* oocysts that are a major source of infection for many intermediate hosts (Dubey *et al.* 2004). As the mainland decline of quolls occurred after the introduction of cats, it is plausible that toxoplasmosis may have been the disease responsible.

While cats have been in Tasmania for over 200 years (Abbott 2008) with no obvious negative effect on eastern quoll populations, several stressors such as drought or habitat loss over recent years may have triggered recrudescence of latent infection into overt disease. Furthermore, if abundance of feral cats increases following devil decline, this would increase the prevalence of the pathogen in the environment, thereby presenting an increased risk of exposure to susceptible wildlife. Indeed, a pilot study in 2010 found higher prevalence of *T. gondii*-specific IgG antibodies at two sites where quolls had declined compared to a site with a stable population (Fancourt 2010). Accordingly, toxoplasmosis is a candidate cause of the recent quoll decline.

1.4.2.4 Foxes

Foxes have been implicated as a major factor in the extirpation of eastern quolls on mainland Australia (Jones and Rose 2001; Jones *et al.* 2003). The pattern of quoll decline broadly coincided spatially and temporally with the fox's geographical range expansion. Eastern quolls fit within the CWR of prey species that have been most affected by foxes on the mainland (Burbidge and McKenzie 1989). However, first-hand accounts of foxes killing quolls are scarce (Peacock and Abbott 2014). Predation (as distinct from scavenging) has been inferred from observations of quoll remains around fox dens (*The Australasian* 9.12.1905: p.1404) or foxes chasing quolls (*The Argus* 11.6.1884: p.3).

However, it seems more likely that disease, rather than fox predation, accounted for the major decline in quoll populations around 1890-1910. An extensive review of historical accounts (Peacock and Abbott 2014) has revealed numerous accounts of quoll decline that predate the introduction or local establishment of foxes (Abbott 2011), and several accounts of quoll hyperabundance postdating fox establishment in some regions. Foxes probably contributed to the final demise of the remaining populations that persisted in low densities for the next 50-60 years. The final descent to a species' extinction is often driven by synergistic processes (amplifying feedbacks) that can be disconnected from the original cause of decline (Brook *et al.* 2008).

Foxes are also unlikely to have been a major contributor to the recent decline of eastern quolls in Tasmania. The fox was recently introduced to Tasmania (Saunders *et al.* 2006; Sarre *et al.* 2012) presenting an imminent threat to a range of CWR species, including the

eastern quoll, should they become established. However, the estimated low density of foxes and the absence of any new fox evidence since July 2011 (Invasive Species Branch 2013) suggests that foxes are likely to be functionally absent from the island.

1.4.2.5 Poisoning

Some poisons with potential to affect the eastern quoll are still in use in Tasmania. Strychnine, cyanide and phosphorus were historically used to poison eastern quolls directly (as predators of domestic poultry) and indirectly (as non-target consumers of rabbit baits), or by secondary poisoning of quolls scavenging carcasses of poisoned rabbits (Lunney and Leary 1988; Peacock and Abbott 2013). While the widespread use of these poisons has now ceased, sodium fluoroacetate (compound 1080) has, in recent decades, been the leading method of strategic control of foxes and wild dogs ubiquitous throughout much of the Australian mainland (Glen *et al.* 2007). In Tasmania, 1080 has been used to control the browsing impacts of herbivores since the 1950s, predominantly delivered as poisoned carrot baits that did not present a significant risk to non-target carnivores (Statham 2005). However, the introduction of foxes around 15 years ago led to the commencement of fox baiting programs in Tasmania in 2002 (Saunders *et al.* 2006). Fox baits initially comprised dried kangaroo meat baits poisoned with 1080, with commercially prepared Foxoff® baits being utilised from around 2006-7 (Nick Bates, Department of Primary Industries, Parks, Water and Environment (DPIPWE) pers. comm.). Both bait types are specifically designed to target carnivores and therefore present a novel risk to the eastern quoll through possible non-target poisoning (McIlroy 1981; 1986; King *et al.* 1989).

For eastern quolls, the LD_{50} of 1.5 mg kg^{-1} (King *et al.* 1989) would mean that an average 0.85 kg female (Godsell 1983) would need to consume less than half of one 35 g Foxoff® bait (3 mg of 1080) to receive a lethal dose, possibly less to kill any nursing young. This is much less than the 90 g of non-poisoned baits consumed in one sitting by eastern quolls in captive trials (Belcher 1998). Laboratory derived sensitivities, however, reflect a given set of variables such as ambient temperature, diet, stress and energy levels unlikely to be reflective of conditions experienced by wild animals in the field (Oliver and King 1983). Accordingly, laboratory trials may provide only weak theoretical evidence of whether 1080 baiting presents a realised risk to species in the landscape (Glen *et al.* 2007).

Eastern quolls may be vulnerable to mortality from fox baits in the landscape, but fox baiting does not appear to have significantly contributed to the recent quoll decline. Field-based sensitivity studies have not been performed for the eastern quoll, however a preliminary review of 1080 fox baiting operations in Tasmania revealed that while quoll declines broadly correlated temporally with the commencement of fox baiting on the island, they did not correlate spatio-temporally. Several quoll populations declined in areas that were either not baited, or were baited several years after quolls had declined in that area (B. Fancourt, unpubl. data).

Rodenticides are another candidate agent in the recent quoll decline. In recent decades, the active ingredient in rodenticides changed from the first generation anticoagulants (FGAs: e.g. warfarin, pindone) to the second generation anticoagulants (SGAs: predominantly brodifacoum and bromadiolone) (Eason *et al.* 2002). During the 1990s, the patent on brodifacoum expired, and its availability and use increased rapidly thereafter (Eason *et al.* 2002). It became widely used in over-the-counter rodenticides that target commensal rodents, but is also increasingly applied in agricultural systems (Eason *et al.* 2002). Rodenticides may result in primary poisoning through unintended ingestion of baits by non-target species, and can also result in secondary poisoning of carnivores that eat poisoned prey or scavenge on their carcasses (Eason and Spurr 1995; Alterio 1996). Brodifacoum in particular has been implicated in increasing numbers of non-target deaths in a range of wildlife species (Eason and Spurr 1995; Stone *et al.* 1999; Thompson *et al.* 2014; Poessel *et al.* 2015). The persistence and potency of the SGAs means that the risk of primary and secondary poisoning from these toxins is greater than that associated with FGAs. In some species, brodifacoum can persist in the liver for more than 8 months (Eason *et al.* 2002). Accordingly, unlike the FGAs, sub-lethal doses of brodifacoum can rapidly bioaccumulate to reach toxic levels, presenting a much higher risk to a range of non-target species. The increasing use of brodifacoum over recent decades and its widespread use in agricultural areas frequented by eastern quolls points to its potential contribution to quoll declines. However, given its widespread use and unrestricted availability (e.g. supermarkets), I am unable to ascertain if increased use of brodifacoum is spatially and temporally associated with the recent quoll decline. Future research should evaluate the risk of such poisoning to eastern quolls.

1.4.2.6 Persecution

Eastern quolls were commonly persecuted throughout recent history, but current persecution levels are unlikely to present a significant threat. Historically, quolls were persecuted as agricultural pests, both on the mainland (Wood Jones 1923; Bennett 1990; Peacock and Abbott 2013) and in Tasmania (Backhouse 1843; Green 1967). Green (1967) considered that when predation on domestic poultry and stock became excessive, population control of quolls (and devils) became a necessary part of "good pasture and stock management". Eastern quolls are now legally protected. There may still be cases of individual quolls being killed, but it seems unlikely that ongoing persecution would be sufficient to have driven eastern quolls to their recent decline.

1.4.2.7 Habitat modification

Certain changes in land use may present a significant threat to quoll habitat availability. While land clearing has been implicated in the historic decline of the eastern quoll on the mainland and in Tasmania (Green 1967; Lunney and Leary 1988), eastern quolls frequently use open areas (Godsell 1983; Jones and Barmuta 2000; B. Fancourt, pers. obs.) and benefit from pasture establishment that is typically accompanied by increases in pasture grubs and agricultural pests such as rodents that form a substantial part of the species' diet (Green 1967; Blackhall 1980; Godsell 1983). However, conversion of agricultural land or natural forest into monocultures such as timber plantation removes either foraging or denning habitat for the species. Tasmania has undergone extensive conversion of large tracts of agricultural and natural vegetation into *Eucalyptus* species plantations during the period of eastern quoll decline (Tasmanian Planning Commission 2009; Forest Practices Authority 2012). Accordingly, habitat modification remains a potential contributor to the recent decline.

1.4.2.8 Road mortality

Eastern quolls are highly susceptible to road mortality, but it is unlikely to be a significant contributor to the recent decline. Quolls often use roads and tracks for long-distance travel, and they opportunistically scavenge roadkills, often becoming casualties in the process (Jones 2000; B. Fancourt, pers. obs.). Road mortality can have a dramatic impact on local quoll populations in a relatively short period of time (Jones 2000). However there

have been no significant expansions of road networks in Tasmania, and therefore while localised losses may still occur, road mortality is unlikely to be a significant contributor to recent statewide declines in the eastern quoll.

1.5 Thesis aims

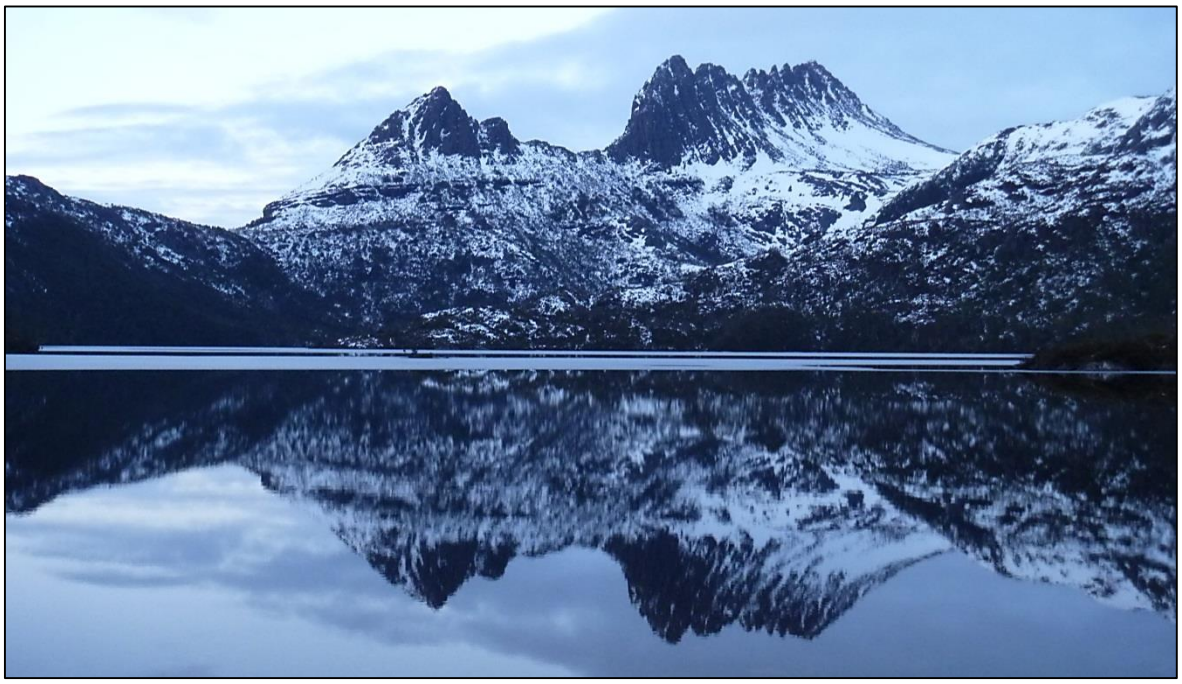
In this thesis, I aimed to identify and investigate key threats and processes that have contributed to the recent precipitous decline of the eastern quoll in Tasmania. First, I developed a dynamic species distribution model for the eastern quoll using short-term weather variables to investigate how temporal fluctuations in quoll abundance compare with variation in the amount of environmentally suitable habitat for the species over time (Chapter 2). Second, I screened eastern quoll populations for the seroprevalence of *T. gondii*-specific IgG antibodies to investigate if *T. gondii* infection differed between sites with declining quoll populations and those with relatively stable quoll populations, and to assess whether acute *T. gondii* infection led to toxoplasmosis or whether latent infection negatively affected quoll survival or reproduction (Chapter 3). Third, I screened feral cats from across Tasmania to investigate if *T. gondii* infection in cats, the parasite's definitive host, differed among regions, thereby contributing to any differing prevalence of *T. gondii* among quoll populations (Chapter 4). Fourth, I used a combination of trapping and camera surveys throughout the eastern quoll's distribution to investigate if the abundance and activity patterns of devils, cats and quolls differed among regions with increasing time since DFTD arrival, and among sites with differing quoll abundance (Chapter 5). Finally, I synthesised my findings to formulate a hypothesis as to the cause of the recent decline of the eastern quoll in Tasmania (Chapter 6). I have designed a future study to test this hypothesis, and to guide in the management and conservation of the species.

1.6 Thesis structure

Each chapter of the thesis is written as a separate article for publication in a different journal, so there is some inevitable repetition of background material, description of study areas and the study species to establish the context for each paper. Where references cited in a published chapter were in press, in review or unpublished at the

time of publication, those references have been updated here to reflect their current status, volume and page numbers. Text formatting and numbering of figures and tables have also been modified here to ensure consistency between chapters. However, all other chapter content here reflects the content of the published article where relevant.

Chapter 2
Testing the role of climate change in species decline: is the eastern quoll a victim of a change in the weather?



Winter at the Cradle Mountain study site, Tasmania (Photo: Bronwyn Fancourt).

In review:

Fancourt BA, Bateman BL, VanDerWal J, Nicol SC, Hawkins CE, Jones ME and Johnson CN (in review) Testing the role of climate change in species decline: is the eastern quoll a victim of a change in the weather? *PLoS ONE*.

2.1 Abstract

To conserve a declining species we first need to diagnose the cause of decline. This is one of the most challenging tasks faced by conservation practitioners. In this study, we used temporally explicit species distribution models (SDMs) to test whether shifting weather can explain the recent decline of a marsupial carnivore, the eastern quoll (*Dasyurus viverrinus*). We developed an SDM using weather variables matched to occurrence records of the eastern quoll over the last 60 years, and used the model to reconstruct variation through time in the distribution of climatically suitable range for the species. The weather model produced a meaningful prediction of the known distribution of the species. Abundance of quolls, indexed by transect counts, was positively related to the modelled area of suitable habitat between 1990 and 2004. In particular, a sharp decline in abundance from 2001 to 2003 coincided with a sustained period of unsuitable weather over much of the species' distribution. Since 2004, abundance has not recovered despite a return to suitable conditions, and abundance and area of suitable habitat have been uncorrelated. We suggest that fluctuations in weather account for the species' recent decline, but other unrelated factors have suppressed recovery.

2.2 Introduction

Detecting, diagnosing and halting species declines are some of the most challenging tasks faced by conservation practitioners (Caughley 1994). Timely action is critical to species recovery (Martin *et al.* 2012). Therefore conservation managers are often forced to act on incomplete knowledge of key threats and factors causing the decline (Soulé 1985). However, unfounded assumptions as to the causal factors can lead to inaccurate predictions of extinction risk and wasted management effort (Ferson and Burgman 1995; Caughley and Gunn 1996). The eastern quoll (*Dasyurus viverrinus*) is a medium-sized marsupial carnivore that was once widespread in south-eastern Australia. The last confirmed sighting on the Australian mainland was in Sydney in 1963 and the species survives only on the large island (68 400 km^2) of Tasmania (McKnight 2008a). In Tasmania, findings from a large-scale monitoring program using transect counts suggest there was a

marked decline in abundance in the early 2000's; this evidence is supported by longitudinal sampling of populations by live-trapping (Fancourt *et al.* 2013). As a result, the species was listed as Endangered under IUCN criteria in the recent Action Plan for Australian Mammals (Woinarski *et al.* 2014). The reasons for this decline are unknown. Population eruptions and declines have been anecdotally reported for the species over more than a century (Peacock and Abbott 2014), suggesting that marked fluctuations may simply be part of the species' natural history. Similar eruptions in rodents have been attributed to short-term changes in rainfall and temperature (Predavec 1994; Lima and Jaksic 1998). If eastern quolls are also sensitive to short-term variations in weather, it is possible that the recent quoll decline may have been driven by a period of unsuitable weather, and that abundance can be expected to recover when conditions return to normal.

Climate exerts a strong influence on the distribution and abundance of many species (Pigott and Huntley 1981; Jiguet *et al.* 2010). Unfavourable climatic conditions may exceed a species' physiological tolerances (St. Clair and Gregory 1990), limit food resources (Thomas *et al.* 1996) or disrupt reproduction and completion of life cycles (Woodward *et al.* 1990). Long-term changes in climatic conditions can gradually erode environmental suitability, leading to asynchronous feeding and breeding cycles (Winder and Schindler 2004) and shifts or reductions in distribution and abundance (Pounds *et al.* 2006; Foden *et al.* 2007). Over shorter time scales, fluctuations in weather and extreme events can cause sudden changes in distribution and abundance (Parmesan *et al.* 2000). For some species, the decline in abundance may be temporary and recovery will ensue without management intervention, while for others it may contribute to a cumulative or permanent trajectory towards extinction (Ehrlich *et al.* 1980; Thomas *et al.* 1996). Many declines due to climate change will probably be stepwise rather than smooth, as the changing climate ushers in extreme weather events that cause abrupt declines. The challenge for conservation managers is to quantify the effects of these short- and long-term climatic changes so that their effects can be measured and distinguished from other possible threatening processes.

Correlative species distribution models (SDMs) use suites of environmental variables to explain observed patterns of species occurrence (Elith *et al.* 2006; Phillips *et al.* 2006; Elith and Graham 2009). Such models are based on the premise that a species' current distribution is a good indicator of the environmental requirements for its persistence (Pearson and Dawson 2003). Climatic SDMs typically use long-term climatic means to define the climatic niche, thereby producing static depictions of distribution that are assumed to be in equilibrium with the current climate (Guisan and Zimmermann 2000). However, by using temporally explicit occurrence and climatic data, weather SDMs provide additional information on changes in the amount and distribution of climatically-suitable space over time (Reside *et al.* 2010; Bateman *et al.* 2012). Such changes are not captured by models using long-term climate means which may not represent the conditions experienced by individuals of short-lived species throughout their lifetime (Zimmermann *et al.* 2009). As the relationship between abundance and environmental suitability is generally positive (Gaston *et al.* 2000; VanDerWal *et al.* 2009b), SDMs that predict temporal variation in the area of suitable habitat for a species may also predict changes in abundance.

In this study, we tested the hypothesis that the recent decline of the eastern quoll in Tasmania is due to short-term variation in climatic variables. We built SDMs for the species using both long-term climate means and short-term weather variables, and we compared the predictions of the area of suitable habitat from the weather model with an index of range-wide abundance of the quoll from standardised transect counts. We made four predictions: (1) climatic variables would provide meaningful predictions of habitat suitability for the eastern quoll; (2) weather SDMs using short-term spatially and temporally explicit weather data would perform better than climate SDMs that use long-term climatic means; (3) weather SDMs would predict a reduction in the amount of suitable habitat corresponding to the period of decline in quoll abundance, and quoll declines would be greatest in regions with lowest habitat suitability; and (4) predicted habitat suitability would exhibit a positive relationship with quoll abundance.

2.3 Materials and methods

2.3.1 Study species

The eastern quoll is widespread in Tasmania but occurs primarily across the drier eastern half of the island (Jones and Rose 1996). It is commonly associated with forest-pasture interfaces that provide open grassland for foraging and adjoining natural forest habitat for denning (Godsell 1983), but also occurs in sub-alpine buttongrass (*Gymnoschoerus sphaerocephalus*) moorlands, sedgelands and a mix of wet and dry sclerophyll forest; however it is absent from large tracts of rainforest (Rounsevell *et al.* 1991; Taylor and Comfort 1993; Fancourt *et al.* 2013). It is predominantly insectivorous, although small mammals, birds, reptiles, blackberries (*Rubus fruticosus*) and other plant matter are also eaten, depending on location and seasonal fluctuations in local prey availability (Blackhall 1980; Godsell 1983; Jones and Barmuta 1998).

2.3.2 Species distribution modelling

We collated 1590 eastern quoll occurrence records from the Tasmanian Natural Values Atlas database (Department of Primary Industries, Parks, Water and Environment 2014b). Records were spread across the time period from 1955 to 2009 and included museum specimens, incidental observations and a range of standardised trapping, spotlighting and camera trap surveys. Observations with date accuracy > 1 month or location accuracy > 10 km were excluded. This ensured that the spatial accuracy threshold for occurrence records was no more than double the resolution of the climatic and weather data (~5 km), thereby reducing the likelihood of covariate errors arising from coarse-resolution observations (Reside *et al.* 2011). To minimise spatial bias from localised survey effort, multiple records within a 5 km radius in the same month and year were treated as a single occurrence record.

Monthly climatic data were obtained at a 0.05° grid scale (~5 km x 5 km) for the period 1947 to 2012 from the Australian Water Availability Project (AWAP) (Jones *et al.* 2009). The spatial resolution of these data was approximately double the maximum home range size for the eastern quoll (Godsell 1983; M. Jones, unpubl. data) and therefore was considered appropriate for this species.

We selected eight climatic variables judged to be relevant to the species' ecology while minimising highly inter-correlated variables. As the species is commonly found in the drier eastern half of Tasmania, we incorporated four precipitation variables derived from the monthly AWAP data (annual precipitation, precipitation of wettest quarter, precipitation of driest quarter and precipitation seasonality measured as coefficient of variation). As insects are a major dietary item for quolls and are affected by environmental temperatures (Chown and Terblanche 2007), we also included four temperature variables (mean annual temperature, maximum temperature of warmest month, minimum temperature of coldest month and temperature seasonality (coefficient of variation)). Long-term climate means for each of the eight variables were calculated for the 30-year period from 1976 to 2005. Around 75% of the quoll occurrence records were contained within this period, thereby ensuring that the recommended 30-year climate baseline closely matched the temporal spread of presence records used to build models (Roubicek *et al.* 2010). Short-term weather variables were calculated for the 12-month and 36-month periods immediately preceding each month, from January 1950 to December 2009. Because the eastern quoll is an annual breeder with a short, synchronised mating season (Godsell 1982), the use of variables calculated for periods less than 12 months was not considered appropriate, as an increase in abundance in response to favourable climatic conditions can occur only once a year. The inclusion of 36-month variables allowed for possible cumulative or lag effects on survival or reproductive success in response to environmental conditions accruing throughout the quoll's 3 to 4 year lifetime (Godsell 1983).

We developed SDMs using the algorithm Maxent (version 3.3.3) (Phillips *et al.* 2006). Maxent uses presence-only records to relate environmental variables to species occurrences on the basis of maximum entropy (Phillips *et al.* 2006). All default settings were used except threshold and hinge features, as this produces more ecologically realistic response curves and provides more general predictions (Austin 2007). Climate models were built by relating the 30-year climate means for each of the eight environmental variables to the occurrence records. Weather models were built by relating both the 12-month and 36-month temporally explicit data for each of the eight environmental variables to the month-year and location of each quoll record. To minimise

the risk of over-fitting, we reduced the number of highly inter-correlated variables by including only one of the 12- or 36-month versions of each variable in the final model (see Supplementary material, Table S1). These were selected based on their respective permutation importance, which indicates the dependence of the model on that variable, normalised to percentages (Phillips 2011). For the final weather model, the 12-month data were selected for annual mean temperature and the 36-month data were used for the remaining seven variables. While there were still some high correlations between the variables used in the final model, the SDM algorithm can handle such correlations (Phillips *et al.* 2004; Elith *et al.* 2006; Elith *et al.* 2010), with all pairwise Pearson correlations between retained variables <±0.85 (Elith *et al.* 2006; Elith *et al.* 2010).

We also converted the default Maxent logistic probability distribution from the final weather model to a binary prediction of suitable/unsuitable habitat using a threshold based on equalising training sensitivity and specificity (Liu *et al.* 2005; Jiménez-Valverde and Lobo 2007). This threshold provided a strict level of discrimination, thereby predicting those areas most likely to represent core habitat for the species, while still predicting a realistic depiction of its known distribution (Wilson *et al.* 2005).

The final weather model was projected onto spatial surfaces consisting of the variables across Tasmania for each calendar month from January 1950 to December 2009, thereby producing a single spatially explicit projection for each month for each of the logistic and binary outputs. The 720 individual monthly projections were then compiled to create a composite static map depicting the geographical distribution of weather-defined suitable habitat for the species.

To account for spatial bias in occurrence records (Reddy and Dávalos 2003; VanDerWal *et al.* 2009a), we replaced the uniform background data with a 'target-group' background created using occurrence records of related marsupial carnivore species. These species would be expected to be captured or observed using the same survey methods as the eastern quoll, and would therefore be drawn from the same sampling distribution (Phillips *et al.* 2009). In this way, the background sample reflected the same bias as our presence data, factoring out sample-selection bias (Dudík *et al.* 2005). The target-group comprised the 1590 eastern quoll records and an additional 6655 occurrence records for

the spotted-tailed quoll (*Dasyurus maculatus*) and the Tasmanian devil (*Sarcophilus harrisii*) sourced as for the eastern quoll records for the time period 1955 to 2009. The total 8245 records were scaled up to create a target-group background consisting of 100 000 random points weighted in direct proportion to both the temporal and spatial distribution of the carnivore occurrence records. The spatio-temporal biases were maintained by drawing from the unique spatial locations with a frequency represented by the empirical unique month-year combination observed. This target-group background was used in all climate and weather models.

We used 10-fold cross-validation to assess model fit (Guisan and Zimmermann 2000). This allowed variance estimates to be calculated and evaluated relative to the mean results of the 10 replicate runs. Model performance was evaluated using the area under the receiver operating curve (AUC) (Elith *et al.* 2006; Phillips *et al.* 2006). The AUC ranges from 0 to 1, where 1 indicates perfect discriminatory ability, 0.5 indicates no better than random and > 0.75 can provide useful discrimination (Elith *et al.* 2006). With presence-only data, the maximum AUC will be < 1 and is smaller for wide-ranging species (Wiley *et al.* 2003; Jiménez-Valverde *et al.* 2008).

2.3.3 Relationship between habitat suitability and abundance

The total annual quoll sightings recorded in the Tasmanian state government's annual vehicle-based spotlight surveys (G. Hocking, Department of Primary Industries, Parks, Water and Environment (DPIPWE), unpubl. data) were used as an index of abundance (AI). These surveys commenced in 1975 to monitor population changes of species subject to harvesting (common brushtail possum *Trichosurus vulpecula*, Tasmanian pademelon *Thylogale billardierii* and Bennett's wallaby *Macropus rufogriseus*), however all non-domestic terrestrial species were recorded (Driessen and Hocking 1992). Each survey was driven along a 10 km transect at a constant speed of 25 km/hr. In 1985, the number of transects was increased three-fold to around 150, and survey protocols were standardised where possible for variables such as observer height from ground, type of spotlight, vehicle survey speed, rain, fog and moon phase to help preserve consistency of data, ensure repeatability, reduce observer bias and increase precision and validity of observations (Southwell and Fletcher 1985). Between 1985 and 1990, new transects were progressively added, providing a larger sample size more representative of the eastern

quoll's distribution (Fancourt *et al.* 2013). While almost 200 transects are currently surveyed between November and February each year, not all transects have been surveyed in all years. Transects are categorised into 29 regions, each containing 3-8 transects grouped by proximity. Due to the extensive spatial coverage across Tasmania, each transect is surveyed only once each year. The lack of replication within each year, together with variability inherent in this type of survey technique, means that the use of this data is restricted to presence only applications, or to long-term trends in abundance. The precision and accuracy is not considered sufficient for assessing short-term changes in abundance at regional or transect scales. While these surveys were designed to monitor species subject to harvesting, they were found to be useful for monitoring long-term trends in other less frequently detected species, including the eastern quoll (Driessen and Hocking 1992) and have been corroborated with trends from trapping surveys for the period 1990 to 2009 (Fancourt *et al.* 2013). Accordingly, these surveys were used for the eastern quoll AI as they provided the broadest spatial coverage of the island, used standardised protocols across years, and were performed around the same time of year annually.

To investigate the spatial relationship between habitat suitability and eastern quoll abundance, regional 10-year changes in the mean AI were overlaid onto the binary core habitat SDM to visually explore whether the largest declines occurred in areas of lowest habitat suitability. For each transect, we compared the mean annual quoll sightings from 1997-99 with those from 2007-09. A 3-year mean was used to reduce the impact of interannual variation in factors that may affect detection probability between years, such as change in observer or differences in time of year or night. The mean annual sightings were then totalled for each region to quantify regional changes in quoll AI over the 10 years to 2009. Regional changes in sightings were previously quantified over this 10 year period in accordance with defined criteria for assessing threatened species status at state, federal and international levels (see Fancourt *et al.* 2013). Only the 150 transects consistently surveyed every year during these two periods were included in the AI for this analysis. As the data precision is not considered adequate for robust quantitative analyses at the regional or transect scale, our assessment was performed using a visual exploratory analysis.

To investigate the temporal relationship between habitat suitability and quoll abundance, we also compared the total quoll AI to the total area of core habitat across Tasmania each year between 1990 and 2009. While 150 transects were used in the regional analysis for the 10 years to 2009, not all of these transects were surveyed every year between 1990 and 2009. Accordingly, for this long-term analysis, we omitted the 3 transects with incomplete data and only included the 147 transects that were surveyed every year during this 20 year period in the quoll AI. As sightings from spotlight surveys were included in the occurrence records used to build all climate and weather models, we derived the amount of environmentally suitable area from a second independent core habitat SDM. The independent SDM was built as outlined for the previous weather model at 2.3.2, however all spotlight survey records were excluded from both the quoll occurrence file and the marsupial carnivore target-group background file used to build the model. In this way, the amount of environmentally suitable habitat derived from this second weather model was independent of the spotlight data used in calculating the AI. The reduced occurrence file excluding spotlight sightings contained 880 eastern quoll records between 1955 and 2009, while the target background file contained a total of 1924 records between 1955 and 2009 for the three carnivore species. Output from this independent weather model was compared to the original weather model to ensure AUC, important variables and geographic distribution did not differ markedly between models. Quoll AI was compared graphically with the total area of suitable habitat from the independent binary output from the weather model from 1990 to 2009. Changepoint analysis was performed using the `'changepoint'` package version 1.1.5 (Killick and Eckley 2014) to identify two key changepoints: (1) the year when mean quoll AI changed, and (2) the year when the relationship between the amount of suitable habitat and quoll AI changed (as defined by the ratio of total suitable area:quoll AI). Changepoint analysis uses a maximum log-likelihood approach to determine the point in a time series where the mean or the variance changes (Eckley *et al.* 2011). For each analysis, we tested for a single changepoint and assumed that the data was distribution-free (Page 1954). The quoll AI was log-transformed to stabilise the variance, and linear regression was used to model the amount of suitable area against the log of quoll AI for each year. Separate regressions were performed before and after the second changepoint and were compared to investigate how the relationship between suitable habitat and quoll AI

changed. All statistical analyses were performed using R (ver 3.0.1, R Development Core Team 2013).

2.4 Results

2.4.1 Distribution models

Both the climate model (mean AUC ± s.d. = 0.774 ± 0.011) and the weather model (Figure 2.1; AUC = 0.755 ± 0.019) provided meaningful predictions of habitat suitability for the eastern quoll. Predictions from both models approximated the species' known distribution in the long-term. While there was no marked difference in model fit, the most important variables differed between models. Precipitation of the driest quarter (37.8%), precipitation seasonality (18.4%) and annual precipitation (15.5%) had the highest permutation importance for the climate model, while precipitation of wettest quarter (38.6%) and minimum temperature of the coldest month (37.0%) were the most important variables for the weather model (Supplementary material, Figure S1). Likelihood of quoll occurrence was negatively associated with all precipitation variables in all models, with highest predicted habitat suitability in areas of low or no precipitation. The minimum temperature of the coldest month was positively related to quoll occurrence at temperatures below 0°C, but negatively related at temperatures above 0°C (Supplementary material, Figure S1).

Performance of the second independent weather model (mean AUC = 0.738 ± 0.014) was consistent with the full weather model. The most important variables and their relationship with likelihood of quoll occurrence did not differ between weather models, with minimum temperature of the coldest month (40.7%) and precipitation of wettest quarter (35.9%) having the highest permutation importance in the second model (Supplementary material, Figure S1).

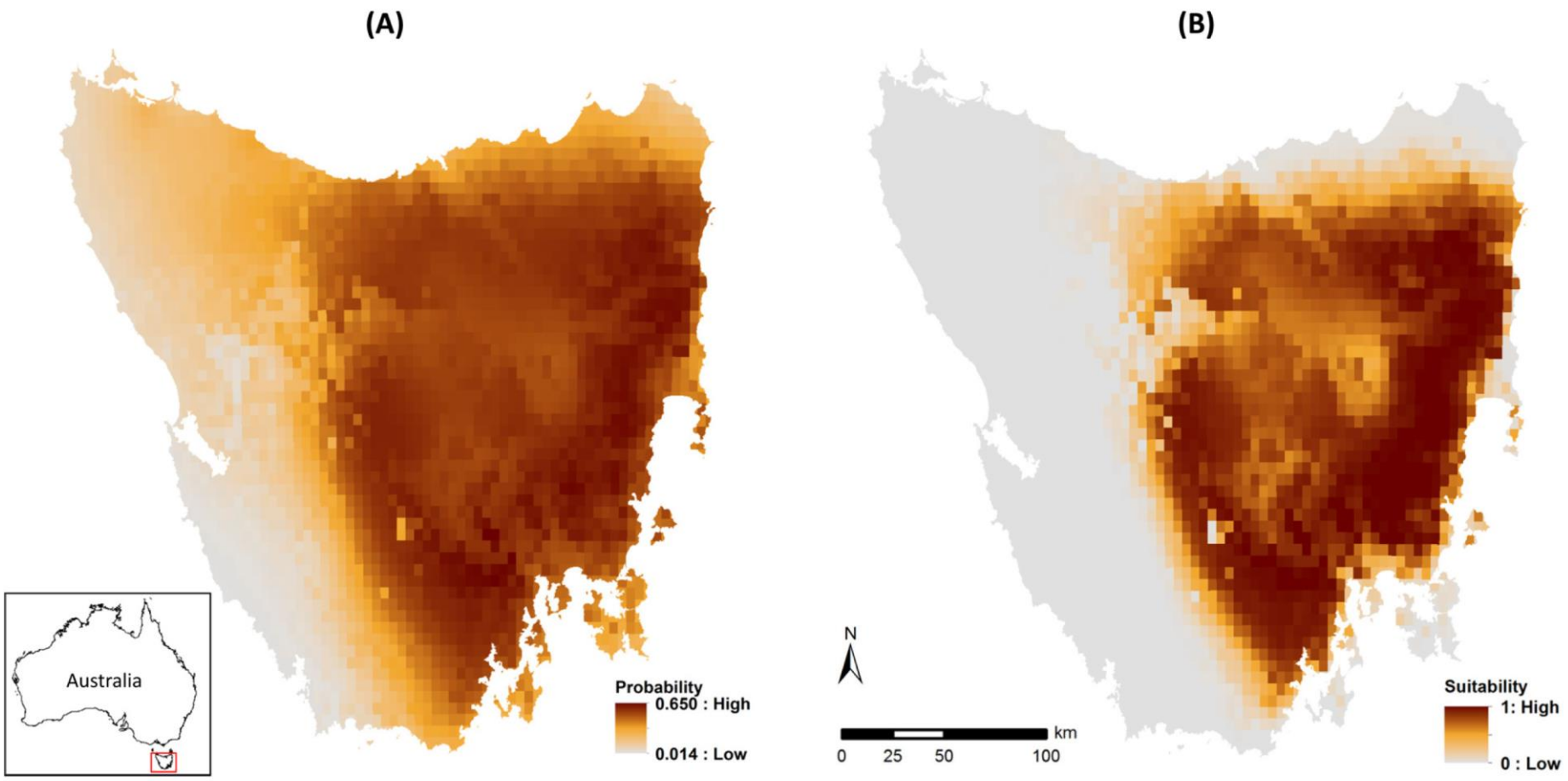


Figure 2.1. Weather-defined species distribution models for the eastern quoll in Tasmania, showing (A) probability of presence (logistic output) and (B) core distribution (binary output). Projections are a composite of the 720 individual monthly projections between January 1950 and December 2009. Grey shading indicates not suitable, with increasing probability or suitability shown from orange to red. Inset shows location of Tasmania within Australia.

2.4.2 Relationship between habitat suitability and abundance

The total area of core habitat fluctuated considerably through time (mean: 29 054 km^2, range: 7200 - 49 625 km^2) (Supplementary material, Video S1). A changepoint in mean quoll AI was identified in 2003, reducing from 56.357 ± 3.591 sightings between 1990 and 2003 down to 31.333 ± 3.242 sightings thereafter. The relationship between suitable area and quoll AI changed one year later in 2004. Temporal trends in the quoll AI were positively correlated with the total amount of core habitat each year between 1990 and 2004 (R^2 = 0.269; $F_{1,13}$ = 4.790; P = 0.047), including a marked decline in both suitable area and AI between 2001 and 2003 (Figure 2.2) when winter minimum temperatures were warmer and precipitation in the wettest quarter was higher. After 2004, quoll AI remained low despite a steady increase in the amount of suitable habitat between 2005 and 2009 (R^2 = 0.010; $F_{1,3}$ = 0.030; P = 0.873) (Figure 2.2).

Spatial patterns of decline in the AI did not match our predictions. The four regions that sustained the greatest decline in abundance in the 10 years to 2009 (76% of island-wide decline in AI over that period) were all located within core areas supporting the highest levels of habitat suitability and stability (Figure 2.3). Regions that experienced the smallest declines in abundance were predominantly located along core habitat margins where habitat suitability was lower (Figure 2.3).

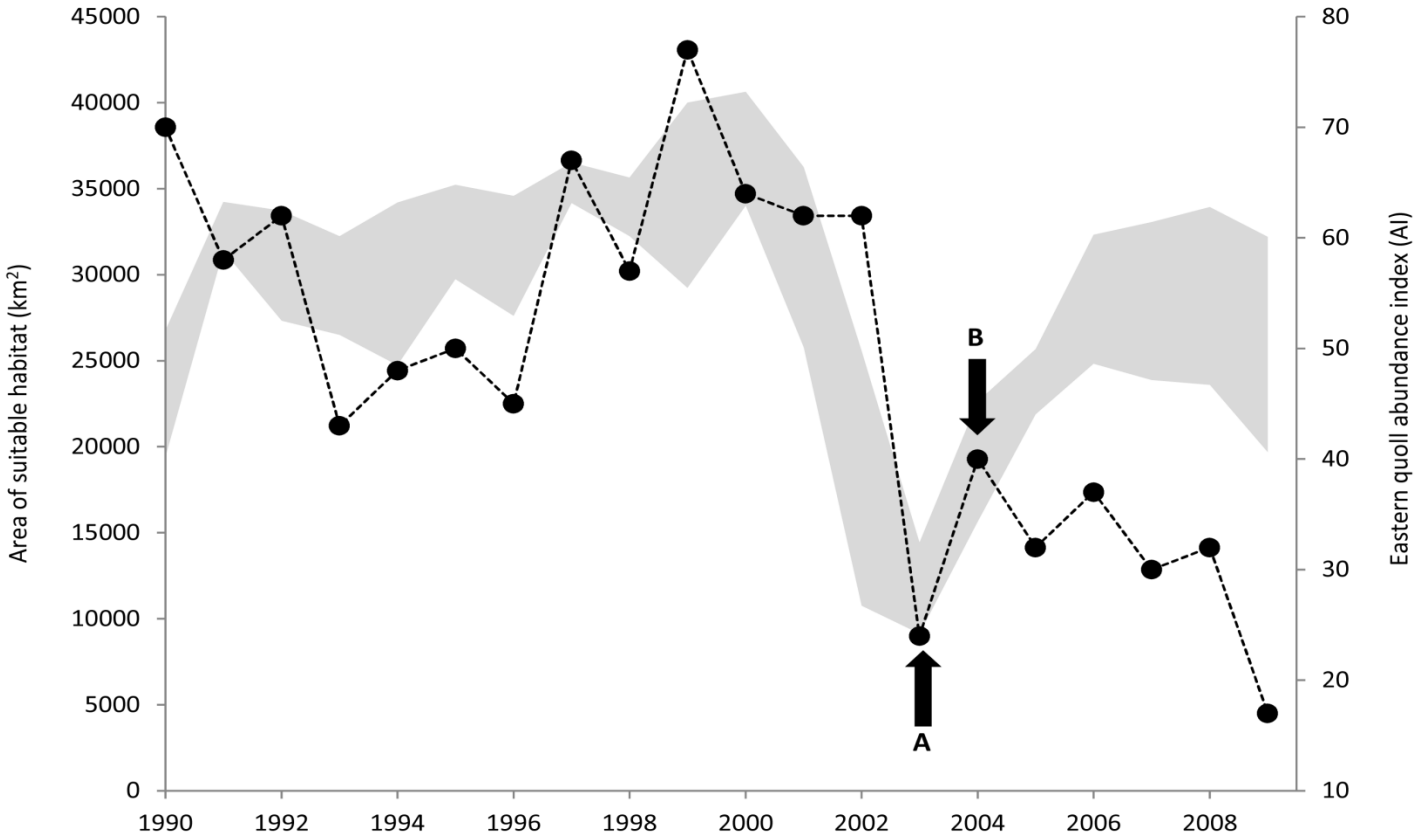


Figure 2.2. Temporal variation in area of environmentally suitable habitat and quoll abundance from 1990 to 2009. Grey shading represents the total area of core habitat across all 12 months for each year (left axis) as given by the independent binary weather model. Width of shading indicates variability of suitable area within each year (lower bound of shading represents the month with the lowest amount of suitable habitat, upper bound represents the month with the highest amount of suitable habitat). Black dots represent the quoll abundance index (AI), being the total number of eastern quoll sightings recorded in annual spotlight surveys across all transects (*n* = 147) surveyed every year from 1990 to 2009 inclusive (right axis) (Fancourt *et al.* 2013). Arrows indicate (A) identified changepoint in mean quoll AI, and (B) identified changepoint in relationship between area of suitable habitat and quoll AI.

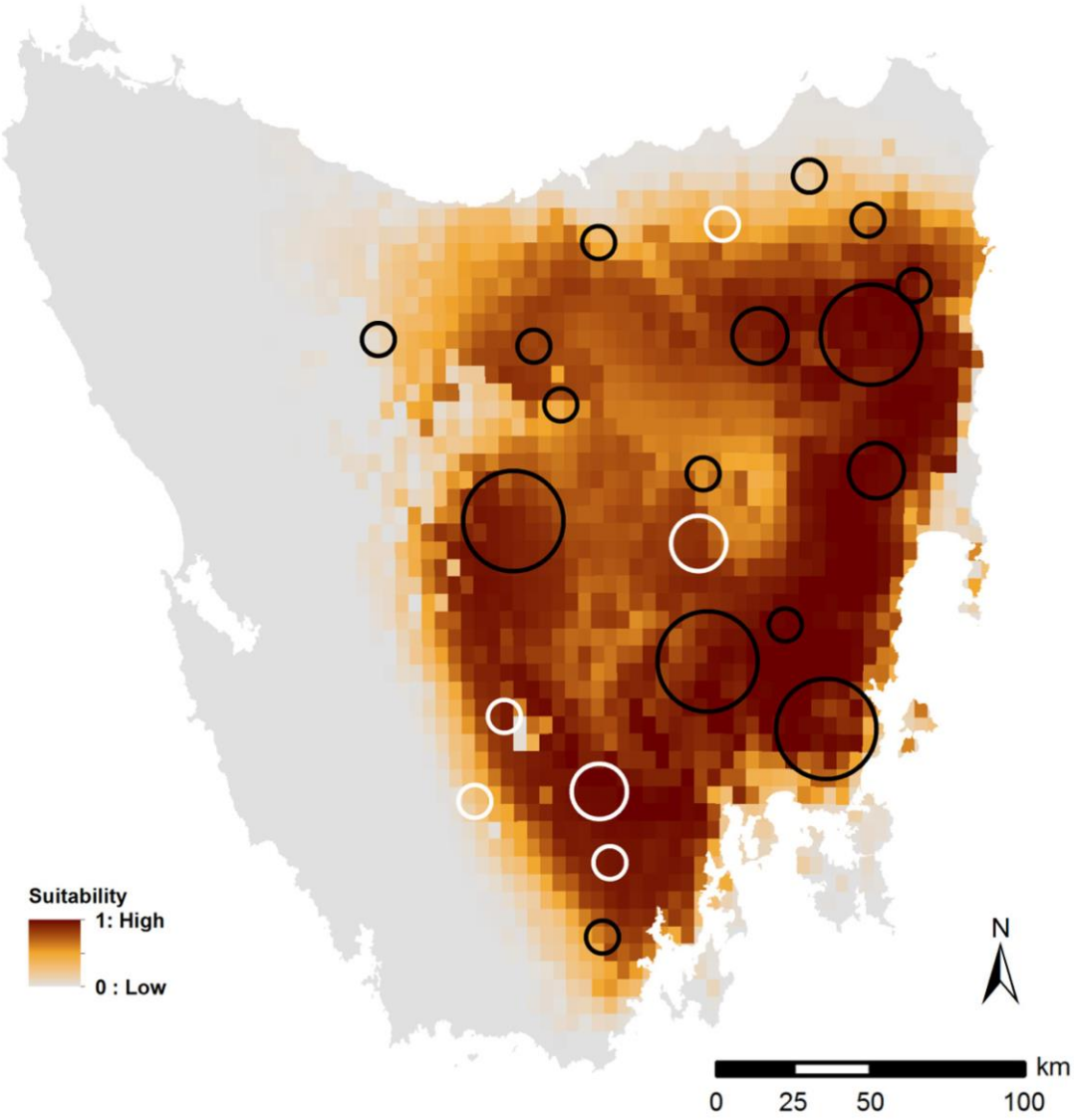


Figure 2.3. Map of Tasmania showing spatial distribution of changes in mean index of eastern quoll abundance (AI) by region over the 10 years to 2009, as recorded in annual spotlight surveys, overlaid onto the predicted core habitat distribution for the eastern quoll as defined by the binary weather model. Change in AI is calculated as the change in the mean annual number of quoll sightings from 1997-99 to 2007-09 for each spotlighting region, based on the 150 transects consistently surveyed in each of these two periods (Fancourt *et al.* 2013). White circles indicate an increase in AI, black circles indicate a decrease in AI for each region. Circle size indicates relative magnitude of absolute increase or decrease in AI, being large circles (>6 quoll sightings), medium circles (3-6 sightings), and small circles (<3 sightings).

2.5 Discussion

We used temporally-explicit weather SDMs to show the contribution of short-term variability in climate to the recent decline of a threatened species. As predicted, fluctuations in abundance of the eastern quoll in recent decades, including a sharp decline between 2001 and 2003, were related to changes in weather across the species' range. More recently this relationship appears to have broken down, however, so that while weather conditions improved after 2004 there has been no corresponding recovery of abundance of eastern quolls. Possibly, the recovery of quolls is now being prevented by some factor unrelated to climate and weather. If so, the recent decline may not be temporary and recovery is unlikely without management intervention.

Both climate and weather models accurately predicted the species' known geographic distribution, suggesting that habitat suitability for the eastern quoll is well characterised by climatic variables. Contrary to our predictions, the discriminative ability and the broader spatial distribution of suitable habitat were similar for both climate and weather models, although differences in suitability were evident at finer spatial scales. This suggests that, when averaged over the 60-year modelling period, weather variables provide similar predictions of long-term habitat suitability to climate models. However, it is the variation within that 60-year period that demonstrates the value of the weather model as an interpretative tool. While climate models provided information on the long-term suitability of habitat for eastern quolls, the weather model revealed how the distribution of suitable habitat varies through time. This short-term variation in habitat suitability is pertinent to conservation managers trying to understand how short-term variation in weather may affect the distribution and abundance of short-lived species, such as the eastern quoll.

Habitat suitability was highest in areas of low precipitation and where minimum winter temperatures fell to around 0°C. Our predicted distribution of core habitat throughout the drier eastern half of the island is broadly consistent with a previous distribution model (Jones and Rose 1996) and matches the species' known distribution. However, the mechanisms by which precipitation and temperature influence eastern quolls require further investigation. It is possible that drier areas support larger populations of the

insects and rodents that form a substantial part of the eastern quoll's diet (Blackhall 1980; Godsell 1983). Minimum winter temperatures may critically influence the species' highly synchronised breeding, suggested by the observation that mating in high-altitude populations occurs up to two months later in years when winter minimums were delayed and warmer (B. Fancourt, unpubl. data). The marked decline in predicted area of suitable habitat during 2001 to 2003 was due to a period of warmer winter temperatures and heavier precipitation. Neither of these shifts was large, but our modelling suggests that in combination, they caused a substantial reduction in suitability of climate for this species. As the frequency of extreme weather events in Tasmania is predicted to increase, specifically warmer temperatures and more intense extreme rainfall events (White *et al.* 2010), our findings highlight an additional long term management concern for the species.

Intraspecific abundance-distribution relationships tend to be positive, such that species declining in abundance also show declines in distribution, and the converse (Venier and Fahrig 1998; Gaston *et al.* 2000). Our analysis is consistent with this, in that our predictions of total suitable area for the eastern quoll through time were positively related to an independent measure of variation in relative abundance, although the strength of this relationship is likely to have been influenced by the severity of the reduction in suitable area during 2003 and 2004. The highest quoll abundance (and subsequently the largest 10-year declines in abundance) occurred in regions with the highest predicted suitability, suggesting that high weather suitability had facilitated the higher abundance prior to the decline. Conversely, the smallest declines occurred at range margins, where population abundance was lower prior to the decline, consistent with the lower habitat suitability in these regions (Hutchinson 1957; Brown 1984).

The wide disparity between suitable habitat and abundance after 2004 indicates that abundance is now being held below its potential value by some factor not included in our weather model. Detailed monitoring using live trapping, camera surveys and additional spotlight surveys at a number of sites between 2010 and 2013 has revealed continuing population declines, with no signs of recovery (Fancourt *et al.* 2013; Fancourt *et al.* 2015 [Chapter 5]). Camera surveys undertaken during 2012-13 confirm that eastern quolls are still widespread (detected at 14 of 17 sites surveyed) across their predicted distribution,

although only low numbers of individuals (between 1 and 4 quolls per linear kilometre) were detected at most sites (B. Fancourt, unpubl. data). This suggests that the current low abundance is not due to a contraction in distribution due to local extinctions, but rather a general reduction of density.

While low environmental suitability, as predicted by SDMs such as Maxent, typically indicates low abundance, abundance may vary over a wide range in areas of high environmental suitability because other factors can affect whether or not potential abundance is realised (VanDerWal *et al.* 2009b). These factors can include habitat type (Rogers and Elliott 2013), competition (Brown 1971), predation (Crooks and Soulé 1999), parasites and pathogens (Pounds *et al.* 2006), dispersal ability (Peterson *et al.* 2001) and disturbance (Woodward *et al.* 1990).

There are a plethora of factors which may be suppressing quoll populations and driving their ongoing decline (Fancourt *et al.* 2013; Fancourt *et al.* 2015 [Chapter 5]). Tasmania is currently undergoing a period of ecological upheaval; the red fox (*Vulpes vulpes*) was recently introduced to the island (Saunders *et al.* 2006; Sarre *et al.* 2012), widespread 1080 fox baiting commenced in 2002 (Saunders *et al.* 2006) and extensive habitat modification and changes in land use have occurred (Forest Practices Authority 2012). The severe decline of the island's largest mammalian carnivore, the Tasmanian devil (Hawkins *et al.* 2006), may be allowing changes in the behaviour and abundance of mesopredators such as feral cats (*Felis catus*) that may threaten a range of species, including the eastern quoll (Jones *et al.* 2007). A recent study found a significantly higher prevalence of *Toxoplasma gondii*, a pathogenic parasite spread by cats, in declining eastern quoll populations than in a comparatively stable population (Fancourt *et al.* 2014 [Chapter 3]). While the parasite did not reduce quoll survival, higher *T. gondii* prevalence signalled higher feral cat activity at the declining sites, suggesting that cats may be contributing to ongoing quoll declines at those sites, possibly through predation, competition or exclusion.

While threats such as feral cats have been present and likely acting on eastern quoll populations in Tasmania for many decades, historic quoll abundance may have been high enough to sustain the impacts of these and other threats without long-term negative

effects on populations. The low quoll abundance observed during 2002-03, however, may have fallen below a critical density threshold from which recovery is difficult or improbable, even in the absence of new threats or increasing severity of existing threats. Small populations are typically more susceptible to extinction through demographic, environmental and genetic stochasticity and natural catastrophes (Shaffer 1981; Caughley 1994; O'Grady *et al.* 2004). Once a species is rare throughout much of its geographic range, the loss of even small numbers of individuals can lead to functional extinction and will rapidly result in local population extinctions (Gaston 2003). In the absence of consistent and reliable abundance records back to 1950, we are unable to determine whether 2002-03 was the first instance of such low abundance of eastern quolls (between 1950 and 2009). However, during this period, the total area of core habitat fell below 15 000 km^2 in only 34 months, with the 18 months from July 2002 to December 2003 representing the longest consecutive period below 15 000 km^2. This unprecedented reduction in core habitat and the historic correlation between core habitat suitability and quoll abundance suggests that the low abundance observed during 2002-03 may also have been unprecedented throughout this 60 year period.

2.6 Conclusion

We have demonstrated that short-term weather variables can influence the distribution and abundance of the eastern quoll. Temporally explicit SDMs related unfavourable weather conditions to a sudden decline in both distribution of core habitat and quoll abundance. However, while improved weather conditions predicted a subsequent recovery in suitable habitat, quoll abundance did not recover. This suggests that the recent decline in abundance is not a short-term fluctuation, and that some unmeasured factor(s) is continuing to suppress quoll populations and inhibit their recovery. We suggest that while the causal agents continue to operate unchecked, ongoing declines may lead to an increased extinction risk. Further research is required to identify these agents.

Chapter 3
Beyond the disease: is *Toxoplasma gondii* infection causing population declines in the eastern quoll (*Dasyurus viverrinus*)?

Eastern quolls on North Bruny Island, Tasmania.

Published as:

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3.1 Abstract

Disease is often considered a key threat to species of conservation significance. For some, it has resulted in localised extinctions and declines in range and abundance. However, for some species, the assertion that a disease poses a significant threat of extinction is based solely on correlative or anecdotal evidence, often inferred from individual clinical case reports. While a species' susceptibility to a disease may be demonstrated in a number of individuals, investigations rarely extend to measuring the impact of disease at the population level and its contribution, if any, to population declines. The eastern quoll (*Dasyurus viverrinus*) is a medium-sized Australian marsupial carnivore that is undergoing severe and rapid decline in Tasmania, its last refuge. Reasons for the decline are currently not understood. Feral cats (*Felis catus*) may be undergoing competitive release following the ongoing decline of the Tasmanian devil (*Sarcophilus harrisii*), with cats suppressing eastern quolls through increased predation, competition, exclusion or exposure to diseases such as toxoplasmosis. To investigate the effects of *Toxoplasma gondii* infection, eastern quoll populations at four sites were regularly screened for the seroprevalence of *T. gondii*-specific IgG antibodies. Seroprevalence was approximately five times higher at sites with declining quoll populations, and there was a negative association between seroprevalence and quoll abundance. However, *T. gondii* infection did not reduce quoll survival or reproduction. Despite a high susceptibility to *T. gondii* infection, eastern quoll populations do not appear to be limited by the parasite or its resultant disease. Significantly higher seroprevalence is a signal of greater exposure to feral cats at sites where eastern quolls are declining, suggesting that increased predation, competition or exclusion by feral cats may be precipitating population declines.

3.2 Introduction

Pathogens, parasites and their associated diseases can have significant negative impacts on wildlife populations, causing reduced abundance (Muths *et al.* 2003; Leroy *et al.* 2004; Hawkins *et al.* 2006), range (van Riper *et al.* 1986; Scott 1988) or even extinction of populations (Thorne and Williams 1988; Cunningham and Daszak 1998; Blaustein *et al.* 2012). Deterministic extinction may result where disease holds mortality rates above replacement rates (Satō *et al.* 1994; Jones *et al.* 2008). Alternatively, disease may suppress fecundity, growth rates or population size, thereby increasing vulnerability to extinction through demographic stochasticity or Allee effects (Caughley 1994; McCallum 1994; Lafferty and Gerber 2002; De Castro and Bolker 2005). Emerging infectious diseases and 'spill-overs' from reservoir animal populations to sympatric wildlife species have increased in recent decades (Daszak *et al.* 1999; Daszak *et al.* 2000; Hawkins *et al.* 2006; Rhyan and Spraker 2010) and are recognised as a key threatening process for many species. However, while infectious disease has been considered among the top five causes of species extinction in the United States (Wilcove *et al.* 1998), it is thought to have contributed to less than 4% of species extinctions worldwide since 1500 (Smith *et al.* 2006). For some of these species, the role of disease in decline or extinction is inferred solely from correlative or anecdotal evidence (Huijbregts *et al.* 2003; Walsh *et al.* 2003; Abbott 2006; Smith *et al.* 2006; Smith *et al.* 2008; Wyatt *et al.* 2008).

To determine the effects of a disease in natural populations, the relationship of disease to survival or fecundity should be established (McCallum and Dobson 1995). While individual clinical case studies may demonstrate a species' susceptibility to a disease (e.g. Canfield and Cunningham 1993; Blanchard *et al.* 2001; Sleeman *et al.* 2009; Eleni *et al.* 2014; Howe *et al.* 2014), correlation between the prevalence of disease or pathogen and population decline does not establish causality. For example, six viruses are known to infect lions (*Panthera leo*) in the Serengeti, but only one, canine distemper virus, clearly decreases lion abundance (Packer *et al.* 1999). Even the presence of a pathogen or parasite in a dying or dead animal provides only circumstantial evidence without demonstrating cause of death (McCallum 1994). In some declining populations, equilibrium prevalence of a benign infection may be high, while some other factor is responsible for the deaths (McCallum and Dobson 1995). However, many studies do not progress beyond

establishing the prevalence of a disease or pathogen in a host population (Gauthier-Clerc *et al.* 2002; Cabello *et al.* 2013; Chadwick *et al.* 2013; Cross *et al.* 2013).

The eastern quoll is a medium-sized Australian marsupial carnivore that is presumed extinct on the Australian mainland, and survives only on the island of Tasmania (McKnight 2008a). Numbers in Tasmania are declining rapidly, with statewide declines of more than 50% in the 10 years to 2009 (Fancourt *et al.* 2013). Population declines are continuing with no sign of recovery (B. Fancourt, unpublished data). The cause(s) of the decline are not currently known. The Tasmanian devil is also in steep decline, due to the spread of the fatal Devil Facial Tumour Disease (DFTD) (Hawkins *et al.* 2006). Devil declines may allow mesopredators such as feral cats to increase in abundance, possibly leading to suppression of eastern quoll populations through increased predation, competition, exclusion or exposure to diseases such as toxoplasmosis.

Toxoplasma gondii is an intracellular coccidian microparasite with a worldwide distribution (Hill *et al.* 2005; Dubey 2010). Infection by *T. gondii* can result in overt clinical disease (Dubey and Frenkel 1972; Innes 1997; Dubey 2010), with fatalities observed in many wildlife species (Work *et al.* 2000; Szabo *et al.* 2004; Jokelainen and Nylund 2012; Howe *et al.* 2014). Some Australian marsupials are especially susceptible to toxoplasmosis (Obendorf and Munday 1983; Canfield *et al.* 1990; Innes 1997; Bettiol *et al.* 2000). In Australia, feral, stray and domestic cats are the only definitive host that can excrete the environmentally persistent oocysts that are the major source of infection for many intermediate hosts (Dubey *et al.* 2004). For around one week following infection, cats shed millions of oocysts in their faeces (Hutchison 1965; Dubey *et al.* 1970b; Frenkel *et al.* 1970; Miller *et al.* 1972; Lukešová and Literák 1998), which can remain infective in the environment for at least 18 months under optimal climatic conditions (Yilmaz and Hopkins 1972; Frenkel *et al.* 1975). Potential intermediate hosts of *T. gondii* include all birds and mammals, which typically acquire the parasite through eating food, soil or water contaminated with the parasite (Miller *et al.* 1972; Attwood *et al.* 1975; Aramini *et al.* 1999; Hill and Dubey 2002). Once eaten, the sporozoites excyst and rapidly multiply as tachyzoites (Frenkel 1973), leading to clinical toxoplasmosis in some hosts. Acutely infected individuals may exhibit a range of clinical signs or symptoms, including lymphadenopathy, anorexia, lethargy, incoordination, apparent blindness, disorientation,

ataxia, dyspnea, icterus, fever, abortion or death (Desmonts and Couvreur 1974; Attwood *et al.* 1975; Tenter *et al.* 2000; Hill and Dubey 2002; Burns *et al.* 2003; Pereira-Bueno *et al.* 2004; Dubey 2010), although pathogenicity and clinical signs vary between individuals and species. However, many immunocompetent individuals remain subclinical (Dubey *et al.* 1988; Hill and Dubey 2002). For individuals that survive acute infection, bradyzoites form latent tissue cysts predominantly in the neural and muscular tissues (Attwood *et al.* 1975; Dubey and Frenkel 1976; Canfield *et al.* 1990). Tissue cysts rarely cause harm and remain *in situ* for the life of the host (Ekanayake *et al.* 2004; Eymann *et al.* 2006), although latent infection has been associated with increases in certain risky behaviours in some species (Hay *et al.* 1984; Webster *et al.* 1994; Berdoy *et al.* 2000; Vyas *et al.* 2007). While infection is commonly acquired through the faecal-oral route, many intermediate host species can transmit the parasite through eating infected animal tissues (Attwood *et al.* 1975; Burns *et al.* 2003), sexually (Arantes *et al.* 2009; de Moraes *et al.* 2010; Santana *et al.* 2013) or congenitally (Beverley 1959; Parameswaran *et al.* 2009).

The hypothesis that toxoplasmosis is contributing to declines of the eastern quoll is plausible for several reasons. First, many aspects of eastern quoll ecology, such as foraging for ground-dwelling invertebrates and scavenging carrion, increases the likelihood of exposure to infective *T. gondii* oocysts and tissue cysts. Second, disease has been implicated in the demise of the eastern quoll on the mainland and in a sudden decline in thylacine, devil and quoll populations in Tasmania in the early 1900s (Wood Jones 1923; Guiler 1961; Green 1967; Peacock and Abbott 2014), with some proposing toxoplasmosis as a candidate disease (Cross 1990; Freeland 1993; Recher *et al.* 1993). Third, while feral cats have been in Tasmania for over 200 years (Abbott 2002) with no obvious negative effect on eastern quoll populations, several stressors such as drought or habitat loss over recent years may have triggered recrudescence of any latent infections into overt disease. However, despite toxoplasmosis posing a significant threat to some Tasmanian mammals (Obendorf and Munday 1983; Skerratt *et al.* 1997; Bettiol 2000) and a high prevalence of *T. gondii* infection in feral cats throughout Tasmania (Fancourt and Jackson 2014 [Chapter 4]), there has been no research investigating the prevalence of *T. gondii* in eastern quolls, nor its effect on population dynamics.

In this study, we address the following four questions. First, is seroprevalence of *T. gondii* associated with population decline of eastern quolls? To answer this, seroprevalence of *T. gondii*-specific IgG antibodies was compared between sites with declining quoll populations and a site with a non-declining population. Individual quolls were screened for clinical signs indicating clinical toxoplasmosis. Seroprevalence was also regressed against quoll captures within a site to identify any negative correlation between seroprevalence and quoll abundance. Second, does survival differ between seropositive and seronegative quolls? We compared recapture data and survival trajectories of seropositive and seronegative individuals within a population. Third, are there indirect effects of *T. gondii* infection on reproduction? We compared annual production of pouch young in females and testicular volume in males during the mating season for seropositive and seronegative quolls. Fourth, which variables that influence exposure to *T. gondii* are associated with differences in seroprevalence within and among populations? We investigated if seroprevalence within a population differed by age or sex of quoll, and if seroprevalence among quoll populations differed with estimated seroprevalence in and activity of feral cats.

3.3 Materials and methods

3.3.1 Study sites

Eastern quolls were surveyed at four study sites in Tasmania: Cradoc (43°06’13”S, 147°02’40”E), Judbury (43°01’24”S, 146°54’50”E), Cradle Mountain (41°38’35”S, 145°57’32”E) and Bruny Island (43°09’48”S, 147°21’17”E) (Figure 3.1). Mean annual rainfall for Cradoc, Judbury and Bruny Island sites ranged from 650 to 740 mm; mean daily minimum and maximum temperatures were 2 and 13°C respectively in winter, and 10 and 22°C in summer. Mean annual rainfall at the Cradle Mountain site was 2830 mm, and mean daily minimum and maximum temperatures were -1 and 5°C respectively in winter, and 4 and 17°C in summer (Australian Bureau of Meteorology 2013 data). Sites were categorised as declining sites (Cradoc and Judbury) or a non-declining site with a high density, stable quoll population (Bruny Island). The population at the Cradle Mountain site fluctuated throughout the study. The population status for each site was

determined during a pilot study undertaken in 2010 by comparing current capture rates to historic studies at each site (Fancourt 2010; Fancourt *et al.* 2013).

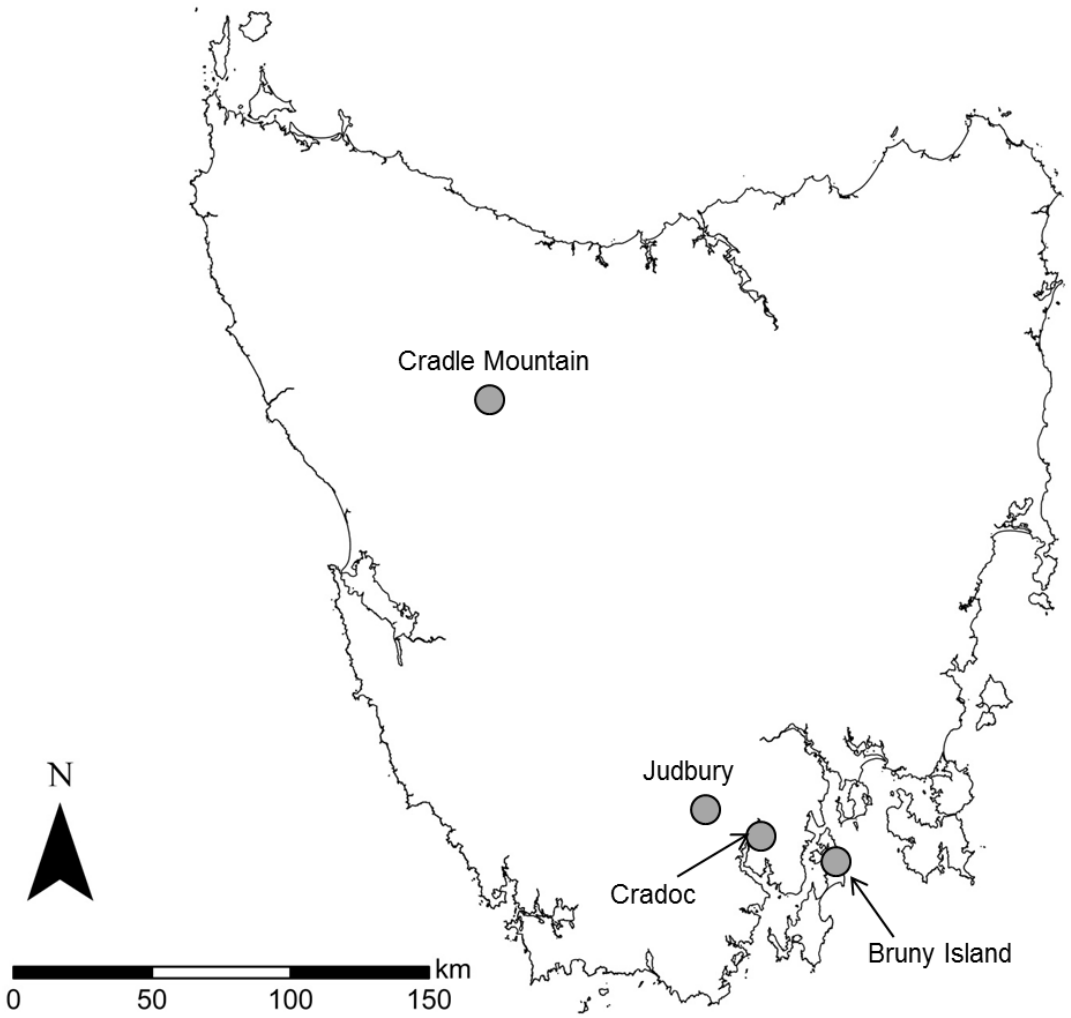

Figure 3.1. Map of Tasmania showing location of study sites used for blood collection.

3.3.2 Quoll surveys, screening and blood sampling

Eastern quolls were surveyed at each site using live capture and release. Sites were surveyed usually every second month from May 2011 to July 2013, although Bruny Island was also surveyed in September 2013 and some prior survey data were available from a pilot study conducted in 2010 at all sites except Judbury. Quolls were captured using standard PVC pipe traps baited with raw lamb heart. All bait was frozen for a minimum of one month at -20°C, then thawed prior to use in traps. This protocol aimed to eliminate the risk of captured quolls acquiring the parasite through eating infected baits (Dubey 1988; Kotula *et al.* 1991; Burns *et al.* 2003). Samples were collected from individual quolls only on their first capture in each sampling period, and were re-sampled if recaptured in subsequent periods. All captured quolls were examined for signs of clinical toxoplasmosis, such as dyspnea, icterus, hind leg paresis, ataxia, and apparent ophthalmic problems. Approximately 300 µL of whole blood was collected from the peripheral ear vein of captured quolls and was kept on ice until processed later the same day. Once clotted, blood was centrifuged for at least 5 minutes and serum frozen at -20°C until processed (within 12 months of collection).

3.3.3 Feral cat surveys and blood sampling

Remote camera surveys were performed to assess feral cat activity at each site. Three replicate surveys were undertaken at each site in February/March 2012, June/July 2012 and December 2012/January 2013, using 20 passive RECONYX™ PC-800 infrared motion detector cameras for a minimum of 21 nights. Each camera was fastened to a tree approximately 1.5 m above the ground, with a muttonbird (*Puffinus tenuirostris*) oil scent lure positioned 2-3 m in front of each camera. Cameras were programmed to take three pictures in rapid succession following each trigger, with images taken continuously in groups of three until all movement ceased. An infrared flash was used to illuminate images at night. All images were stamped with the time, date, site and camera number.

Blood was collected from 55 feral cats trapped, euthanased and frozen on Bruny Island under control programs conducted by the Tasmanian Parks & Wildlife Service, and from an additional six cats trapped and immediately euthanased at the Judbury study site as part of this study. For 23 of the Bruny Island cats, samples were collected from cats

defrosted up to 2 years later. For the Judbury cats and 32 of the Bruny Island cats, blood was collected using cardiac puncture soon after death. All blood samples were processed and stored as outlined in section 3.3.2.

3.3.4 Testing for *T. gondii* IgG antibodies

Serum samples were defrosted and tested for the presence of *T. gondii*-specific IgG antibodies using a commercial modified agglutination test (MAT) (Toxo-Screen DA, bioMérieux, Marcy-l'Etoile, France). IgG antibodies are usually detectable within 2 weeks of initial infection and remain detectable for the life of the host (Remington *et al.* 2004; Dubey 2010). Accordingly, MAT-derived titres are not indicative of recency of infection or clinical status (Dubey 2010) but rather an exposure to the parasite at some time at least 2 weeks before sampling. Of the agglutination tests that do not require species-specific reagents, MAT is considered to be the most sensitive for detecting *T. gondii* specific-IgG antibodies in marsupials (Munday 1972; Dubey 2010). Haemolysis does not interfere with the test, so it can be used with serum, blood plasma or even whole blood (Dubey 2010).

Samples were treated with 2-mercaptoethanol to denature any IgM antibodies and suppress any non-specific agglutination (Desmonts and Remington 1980; Dubey and Desmonts 1987). Each sample was tested at serial four-fold dilutions of 1/16, 1/64 and 1/256 together with positive and negative controls supplied in the MAT kit. A positive reaction was observed when agglutination of toxoplasma formed a mat covering about half of the well base. Titres were expressed as the inverse of the highest dilution at which a positive reaction was observed. A titre of ≥64 was used for determining a sample as positive for *T. gondii* infection (Dubey and Desmonts 1987).

To validate the results obtained using these protocols, a sub-sample of sera underwent retesting by the Tasmanian government Animal Health Laboratories. Where longitudinal samples were collected from individual quolls over multiple sampling periods, further validation was obtained by checking that seroconversion occurred only once in each quoll's life, and that seroconversion occurred only in one direction (from seronegative to seropositive).

To validate the reliability of results using blood from frozen cats, 20 samples were collected from cats at the time of death in 2012, and matched to samples from the same cats after the body had been frozen for around 12 months.

3.3.5 Data analysis

All statistical analyses were performed using R (ver. 3.0.1, R Development Core Team 2013).

3.3.5.1 Seroprevalence

Quolls were classified as adults by May of the year following birth (when they were 10-11 months old), as both sexes reach sexual maturity by this age (Bryant 1986). Seroprevalence was calculated as the proportion of quolls tested in each sampling period that were seropositive. We used a Fisher's exact test to determine if adult seroprevalence at the declining sites was significantly different from that at the non-declining site. As several individual quolls were sampled in multiple periods (but not every quoll in every period), seroprevalence was calculated and compared for each sampling period separately. Any increase in type I error resulting from multiple comparisons was considered unimportant due to the highly significant *P*-values in every period, and was unavoidable due to the non-independence of individual quolls between sampling periods. Because of the high number of periods with 100% prevalence at the declining sites and the resultant infinite odds ratios in each period, a generalised linear mixed model could not be used for this analysis. Only those sampling periods between May 2011 and July 2013 where both declining and non-declining sites were sampled were included in the analysis.

To identify whether seroprevalence in juveniles and the rate of seroconversion differed between declining sites and the non-declining site, seroprevalence was compared and assessed graphically for each annual juvenile cohort (2011 and 2012 emergence), from time of first emergence in November until September of the following year.

To investigate whether increased seroprevalence was correlated with decreased quoll abundance within a site, seroprevalence for each sampling period was regressed against the number of quolls captured in the subsequent survey period (2 months later) as an index of abundance. This analysis was restricted to data from Cradle Mountain as it was

the only site where seroprevalence fluctuated throughout the study, enabling the number of captures to be compared at differing levels of seroprevalence in different periods. Seroprevalence for each period was taken as the number of seropositive quolls captured in that period plus the number of quolls known to be seropositive at that time (but not captured in that period) divided by the total number of quolls known to be alive in that period. Eastern quoll capture data were square root transformed to stabilise the variance, and linear regression was used to model seroprevalence against the square root of the number of quolls captured in the subsequent survey period.

3.3.5.2 Recapture and survival

Data from the Bruny Island site were used to assess recapture likelihood and survivorship. This was the only site with sufficient numbers of both seropositive and seronegative individuals captured in every sampling period, and allowed the effect of serological status to be assessed without involvement of other confounding variables that might be contributing to declines at other sites and that might differ among sites.

The proportion of individuals recaptured was compared between serological groups to identify any effect of serological status on recapture likelihood. All individuals were included in the analysis except those first captured in the final trapping session in September 2013 as there was no possibility of recapture data. Juveniles first captured between November and March each year and not recaptured were also excluded as a high rate of juvenile dispersal is typical in this species soon after first emergence from the den in summer each year (Godsell 1982; Bryant 1986), so failure to recapture these individuals could be due to dispersal rather than death. The proportion of seropositive and seronegative individuals recaptured was compared using a Fisher's exact test.

All individuals first captured between August 2010 and October 2012 were included in the survival analysis, with recapture data up to September 2013 used to assess survival of each individual.

Quolls first captured after October 2012 were excluded due to insufficient time to ascertain robust survival data between first capture and the end of the study in September 2013. Juvenile quolls that were first captured during the period of juvenile emergence and not subsequently recaptured were also excluded. The number of days

known to be alive was used as a measure of quoll survival time, and was calculated from the date of birth (estimated from 1 July in the year of birth) to the most recent capture for each quoll. As the ultimate fate of each individual was not known, analysis was performed on censored data, with failure to recapture an individual assumed to be failure to survive at the date of last capture. Mean survival time was compared between seropositive and seronegative quolls using a one-way ANOVA, and Kaplan Meier (KM) survival curves were used to compare the survival of seropositive and seronegative individuals throughout the study period. A log-rank test was used to identify differences between KM survival curves, with an average hazard ratio calculated to provide an overall comparison of the two serological groups. To quantify effects of serological status on mean longevity, survival time for the oldest cohort (all quolls born in 2009 or earlier) was compared between serological groups using a one-way ANOVA.

3.3.5.3 Reproduction

The mean number of pouch young (PY) in July was compared between seropositive and seronegative females using a three-way ANOVA incorporating site and quoll age. Females from all sites and all years were included in the analysis. Only 2 quolls were captured in July in more than one year; data from their second year were excluded. Females at Cradle Mountain bred around 2 months later than other sites in most years, so were assessed in either July or September, depending on when PY first appeared at that site.

Testicular volume (TV) was calculated for each male quoll captured in May (the mating season) using the formula for a prolate spheroid: TV (cm^3) = 0.5236 x TL x TW^2 (Bailey *et al.* 1998; Power *et al.* 2009). Mean TV was compared between seropositive and seronegative males using a two-way ANOVA incorporating age of quoll at the time of assessment. Males from all years were included in the analysis. Where individual quolls were captured in May in more than one year, data were included only from the first (for seropositive males) or second year (for seronegative males). As the likelihood of infection increases with age (due to increased exposure over time), excluding data from the first capture for seronegative males reduced the likelihood of inadvertently biasing younger males in the seronegative sample set. Males from the Cradle Mountain site were excluded due to an unpredictable delay in breeding at this site in some years, meaning assessment of May TV did not indicate breeding condition in some years.

A body condition index (BCI) was calculated for each female and male at the same time reproductive condition was assessed. Body mass was regressed against maximum head width for each sex, and the regression was used to predict body mass from the observed head width for each individual. BCI was calculated for each quoll as the ratio of observed to predicted body mass (Krebs and Singleton 1993). BCI was compared between seropositive and seronegative individuals, separately for each sex, using a three-way ANOVA incorporating site and quoll age (females) and a two-way ANOVA incorporating quoll age (males).

3.3.5.4 Exposure variables

To investigate whether seroprevalence differed between sexes, we used a Fisher's exact test to compare seroprevalence between adult male and female quolls for each sampling period. Only quolls from the non-declining site (Bruny Island) were used in this analysis as it was the only site with both seropositive and seronegative individuals of both sexes in most periods.

To determine if *T. gondii* infection was affected by quoll age, we used a generalised linear mixed model (GLMM) fit by maximum likelihood with a binomial error distribution and logit link function using R package `lme4` (Bates *et al.* 2013). Individual ID was treated as a random factor to account for non-independence of individual quolls between sampling periods. The model was fit with site (non-declining, declining or Cradle Mountain) and quoll age as fixed effects. All quolls of all ages were included in the analysis, with probability by age plotted for each site.

An index of cat activity was calculated for each site by dividing the number of feral cat detections by the number of camera nights for each camera survey. The mean cat detection rate per 100 camera nights across all 3 surveys was then compared using a two-tailed *t*-test to identify any difference in cat activity among sites.

Seroprevalence in cats was compared using a Fisher's exact test to identify if infection rates differed between sites.

3.4 Results

No signs of overt toxoplasmosis were observed in any of the 290 quolls captured and examined on 1138 occasions between March 2010 and September 2013.

3.4.1 Seroprevalence

Declining sites had significantly higher seroprevalence (range: 77.3 - 100.0%) than the non-declining site (range: 9.4 - 29.4%) in every period throughout the study ($P < 0.005$ for all periods) (Figure 3.2). There were no differences among sampling periods. Seroconversion of newly emerged juveniles occurred earlier and more rapidly at declining sites than at the non-declining site (Figure 3.3), being evident by January for both cohorts at the declining sites, but not evident at the non-declining site until July 2012 or May 2013 (2011 and 2012 cohorts respectively). All juveniles were seropositive at the declining sites by May 2012 or July 2013 (2011 and 2012 cohorts respectively) while seroprevalence at the non-declining site was still below 10% almost a year after emergence for both cohorts. There was a significant negative association between seroprevalence and the number of quolls captured two months later at the Cradle Mountain site (adjusted $R^2 = 0.393$, $F_{1,10} = 8.128$, $P = 0.017$) (Figure 3.4).

3.4.2 Recapture and survival

The serological status of the 151 quolls captured at the non-declining site had no effect on the likelihood of recapture ($P = 1.000$): 61.9% (95% CI: 40.1 - 83.7%) of seropositive quolls and 60.8% (95% CI: 52.0 - 69.5%) of seronegative quolls were recaptured in at least one subsequent trapping session. Mean survival time did not differ significantly with serological status ($F_{1,85} = 2.018$, $P = 0.159$). For first-captures prior to October 2012, seropositive quolls ($n = 11$) survived 890.5 ± 92.8 (mean ± s.e.) days compared to 758.1 ± 32.8 days for seronegative quolls ($n = 76$). Mean longevity of the oldest cohort did not differ between serological groups (seropositive: 949.0 ± 149.6 days, seronegative: 953.6 ± 52.4 days; $P = 0.972$). KM curves indicated a similar survival trajectory for both serological groups ($P = 0.261$) (Figure 3.5). The mean hazard ratio (or conditional failure rate) comparing seronegative to seropositive quolls of 1.17 indicated no relationship between serological status and survival.

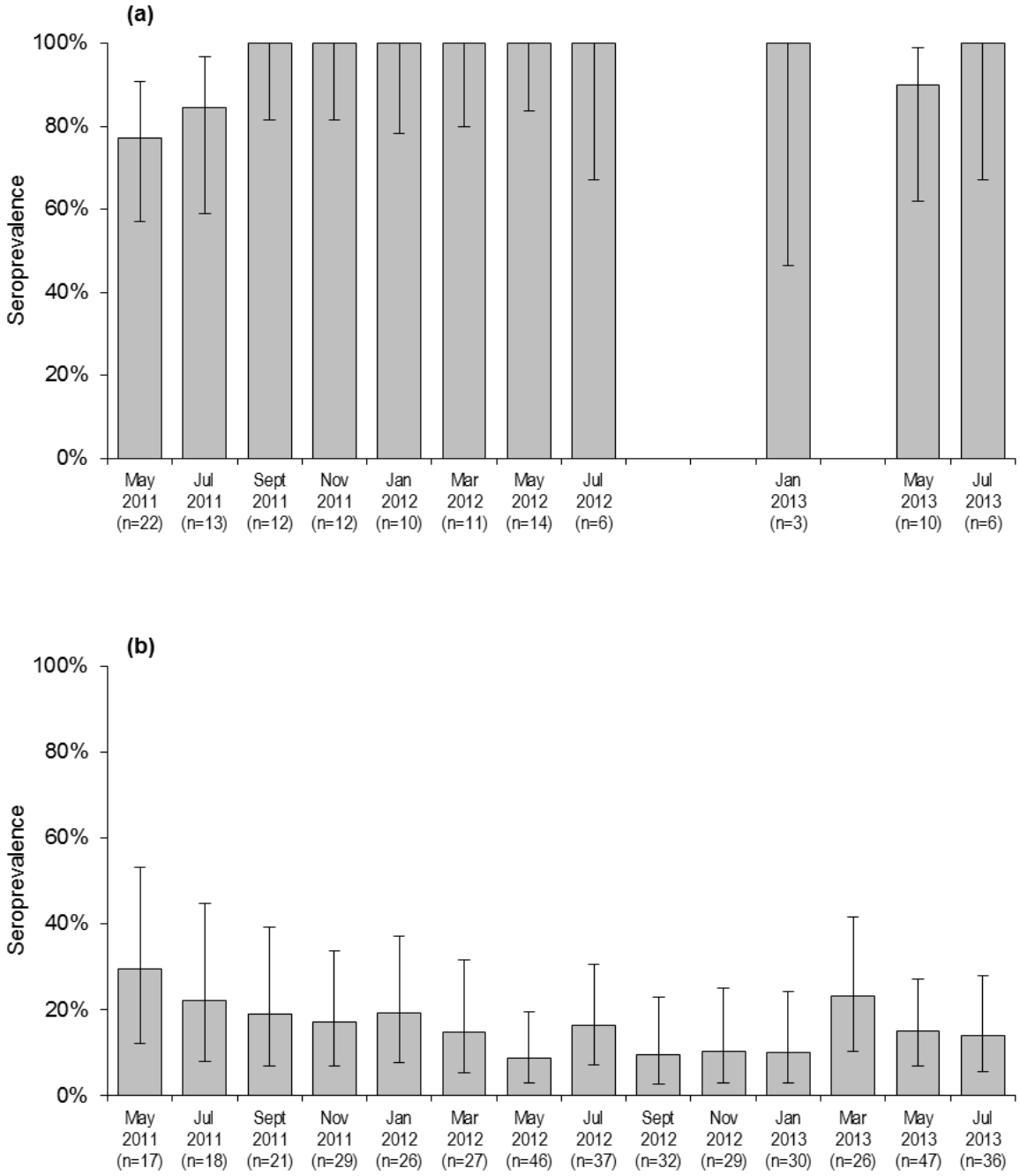

Figure 3.2. Seroprevalence of *T. gondii* IgG antibodies in adult eastern quolls at (a) declining sites (Cradoc and Judbury) and (b) non-declining site (Bruny Island). Declining sites were not surveyed in September or November 2012 or March 2013. Vertical axis shows proportion of quolls tested that were seropositive at titres ≥ 64. Error bars represent 95% confidence intervals calculated using the Jeffreys interval estimation for a small sample size with binomial distribution (Brown *et al.* 2001).

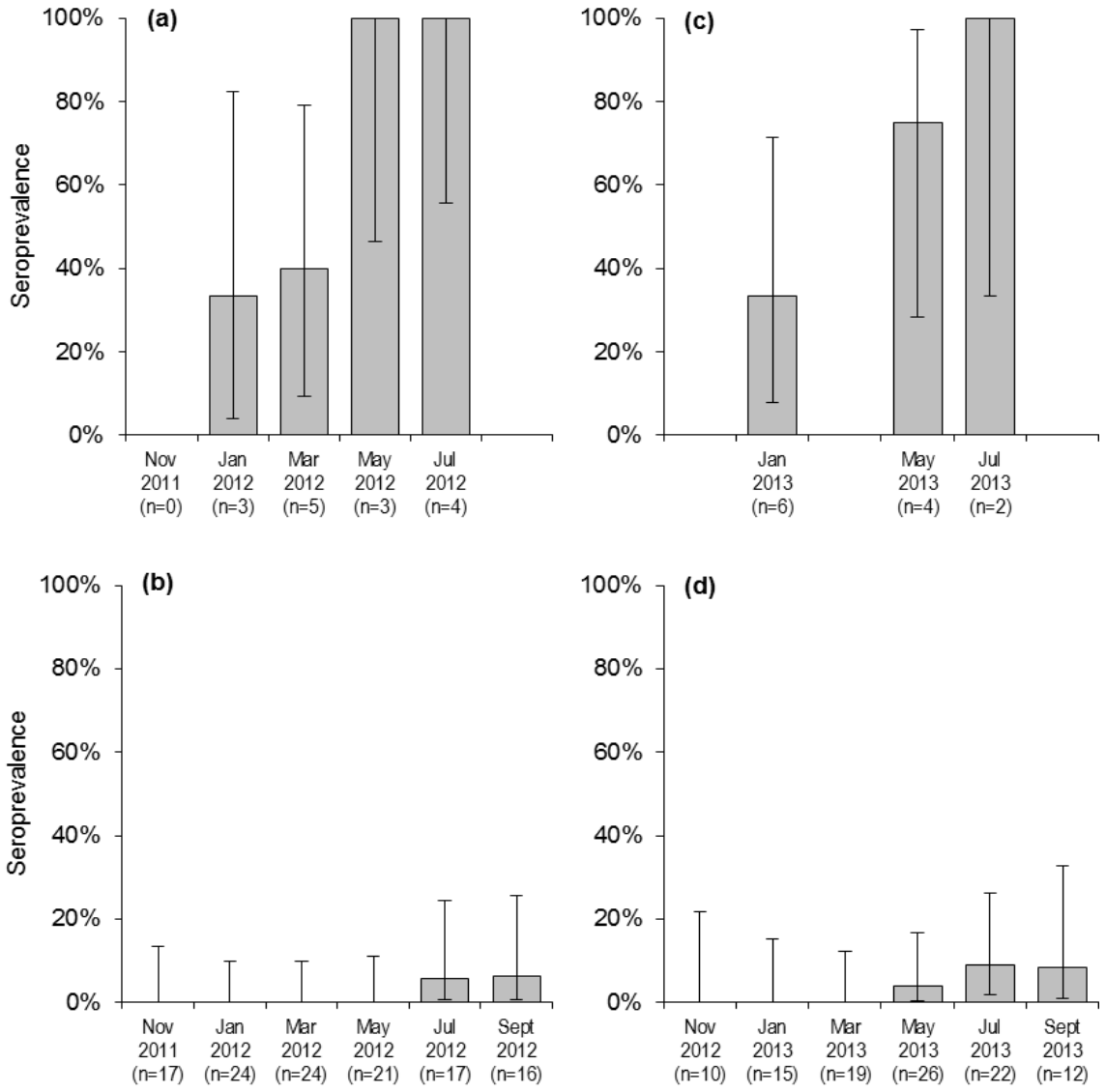

Figure 3.3. Seroprevalence of *T. gondii* IgG antibodies in juvenile eastern quolls from time of emergence for 2011 cohort ((a) declining sites (Cradoc and Judbury) and (b) non-declining site (Bruny Island)) and 2012 cohort ((c) declining sites and (d) non-declining site). Declining sites were not surveyed in September 2012, November 2012, March 2013 or September 2013. Vertical axis shows proportion of quolls tested that were seropositive at titres ≥ 64. Error bars represent 95% confidence intervals calculated using the Jeffreys interval estimation for a small sample size with binomial distribution (Brown *et al.* 2001).

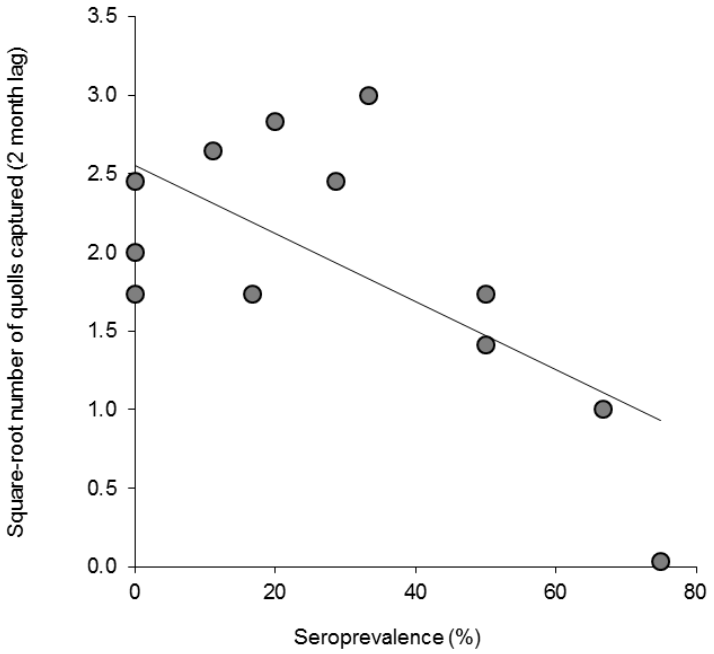

Figure 3.4. Association between seroprevalence of *T. gondii* IgG antibodies in eastern quolls at Cradle Mountain and the square-root transformed number of eastern quolls captured 2 months later (y = 2.552 - 0.022x). Each data point represents a single trapping/sampling session between May 2011 and July 2013.

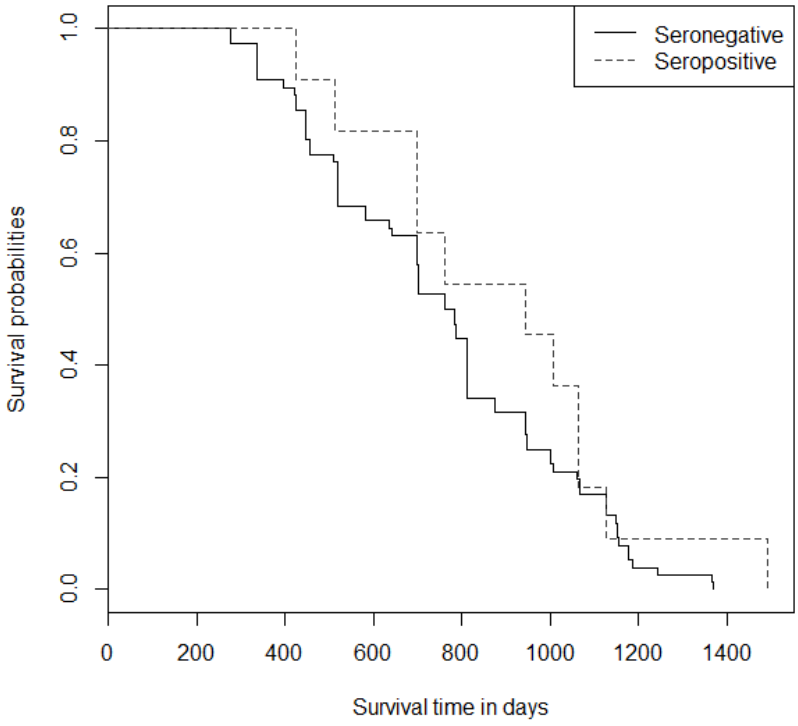

Figure 3.5. Kaplan Meier survival curves comparing survival trajectories for seronegative (solid line) and seropositive (broken line) eastern quolls. Curves show survival of all quolls first captured at the non-declining site between August 2010 and October 2012.

3.4.3 Reproduction

Seropositive females had significantly more pouch-young (6.0 ± 0.0) than seronegative females (4.0 ± 0.4) ($F_{1,30}$ = 7.101, P = 0.012). There was no effect of site ($F_{2,30}$ = 2.002, P = 0.153) or age of mother ($F_{1,30}$ = 0.839, P = 0.367) on number of pouch-young. Body condition did not differ with serological status ($F_{1,21}$ = 0.680, P = 0.419) or age of mother at time of PY assessment ($F_{1,21}$ = 0.471, P = 0.500), however BCI was significantly higher at the non-declining site than other sites ($F_{2,21}$ = 4.517, P = 0.023).

Mean testicular volume ($F_{1,87}$ = 9.473, P = 0.003) and body condition ($F_{1,87}$ = 9.945, P = 0.002) were both significantly higher in seropositive males. While age had no effect on mean TV ($F_{1,87}$ = 0.126, P = 0.723), BCI was significantly higher in older males ($F_{1,87}$ = 8.328, P = 0.005).

3.4.4 Exposure variables

Seroprevalence in male quolls (range: 11.1 - 35.7 %) was higher than in females (range: 0.0 - 3.7 %) in all periods, however differences were not significant in any period except May 2013 (P = 0.010). The probability of *T. gondii* infection increased with age, with a significant interaction between age and site (P < 0.001) (Figure 3.6). At any given age, the probability of infection was significantly higher for quolls at the declining sites than either the non-declining site or Cradle Mountain (P = 0.034).

The mean rate of cat detections (per 100 camera nights) of 1.9 ± 0.3 was significantly higher at the declining sites than the 0.1 ± 0.1 detections at the non-declining site (T_4 = 6.457, P = 0.003). One cat was detected at the Cradle Mountain site in the March 2012 camera survey (0.2 detections per 100 camera nights) but no cats were detected in either the July 2012 or January 2013 surveys.

Seroprevalence of feral cats from Bruny Island (non-declining) was 80% (44/55; 95% CI: 68.1 – 88.9%) compared with 100% (6/6; 95% CI: 67.0 – 100.0%) of cats captured at the Judbury (declining) site, although differences were not significant (P = 0.577). No cats were captured at either the Cradoc or Cradle Mountain sites. Titres obtained from frozen cats matched titres from fresh samples collected at the time of death prior to freezing, indicating no difference in results due to sample type (fresh or frozen).

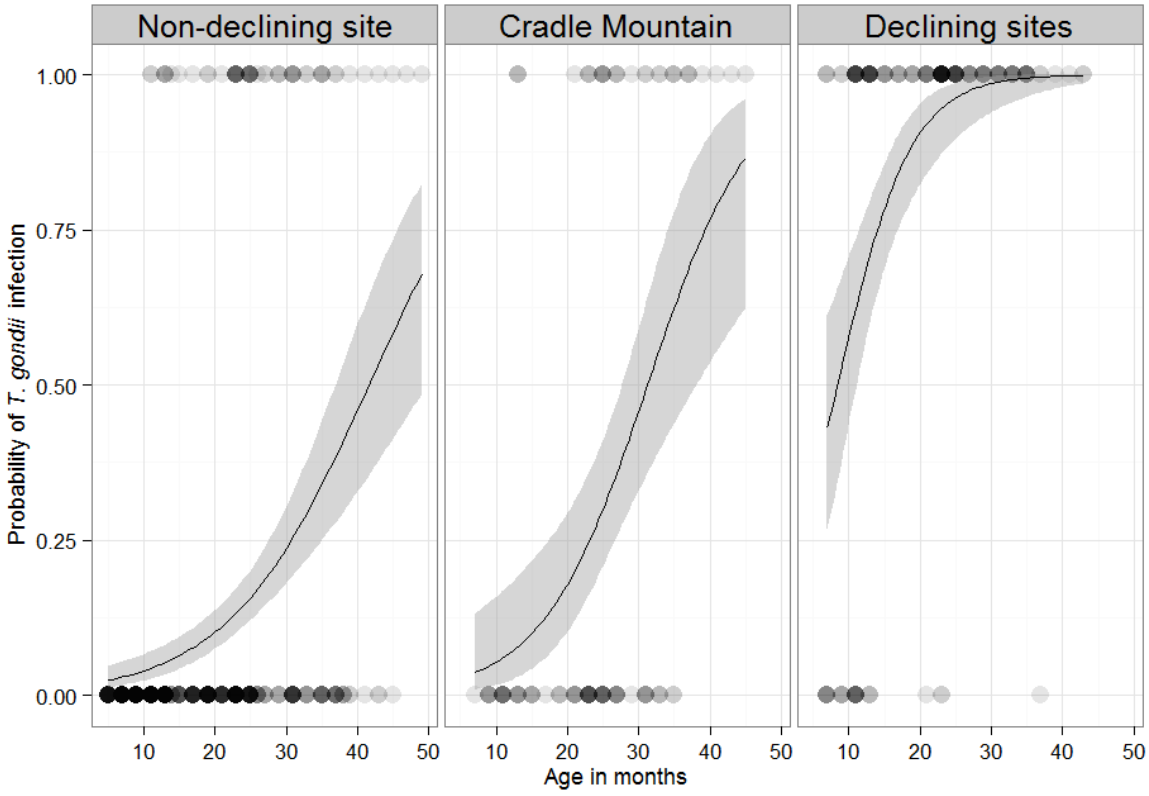

Figure 3.6. Comparison of the probability of *T. gondii* infection with quoll age, by site. Non-declining site = Bruny Island, Cradle Mountain = fluctuating site, Declining sites = pooled data from Cradoc and Judbury sites. Circles represent individual observations of seronegative (probability = 0) and seropositive (probability = 1) quolls at a given age, with darker circles indicating a higher number of quolls with the same combination of age and serological status. Curve illustrates the fitted data, with grey shading representing 95% confidence intervals.

3.5 Discussion

We found no evidence that *T. gondii* infection reduces survival or reproduction of eastern quolls. Seroprevalence of *T. gondii* antibodies was higher at sites with declining quoll populations than in the non-declining population, and there was a negative association between seroprevalence and the number of quolls captured. While this might suggest a causal link between *T. gondii* infection and quoll declines, our epidemiological studies suggest no such link. High prevalence *per se* is a poor indicator of the impact of disease on a population (McCallum and Dobson 1995). On the one hand, highly virulent diseases remove infected individuals soon after exposure, either through rapid death or predation of symptomatic individuals, leaving only unexposed individuals to be detected and a low observed prevalence (e.g. Obendorf *et al.* 1996). On the other hand, if a disease is benign, infected individuals remain to be detected and the observed prevalence of the disease will be relatively high (McCallum 1994). This is evidently the case for *T. gondii* in the eastern quoll.

The nonpathogenicity of *T. gondii* in eastern quolls is also supported by the absence of clinical signs in any of the quolls captured and examined in this study. Tasmanian government Animal Health Laboratory records also have no cases of histopathology indicating clinical toxoplasmosis in any seropositive quolls examined (B. Jackson, Department of Primary Industries, Parks, Water & Environment (DPIPWE), pers. comm.), although we acknowledge that given the unspecific clinical signs associated with the disease, diagnosis is challenging to reach both ante mortem and post mortem in many host species. An extensive search of the literature (this study; D. Peacock and I. Abbott, pers. comm.) uncovered only one suspected case of toxoplasmosis in what was probably an eastern quoll (‘native cat’ *Dasyurus quoll*: Carne (1950) unpubl. data, cited in Seddon (1952)). However this was based on necropsy findings of “toxoplasms” in the lung of a moribund individual, probably bradyzoites associated with dormant tissue cysts and not diagnostic of clinical disease. Reasonably high seroprevalence has been recorded in many marsupial carnivore species, including spotted-tailed quolls (*Dasyurus maculatus*) (Hollings *et al.* 2013), western quolls (*Dasyurus geoffroii*) (Haigh *et al.* 1994) and Tasmanian devils (Phillips 2009; Hollings *et al.* 2013), but with no known confirmed cases of clinical toxoplasmosis. Therefore, while the high seroprevalence indicates that the

larger marsupial carnivores are highly susceptible to *T. gondii* infection, they are evidently less likely to succumb to acute disease than other marsupial guilds.

The lower pathogenicity of *T. gondii* in marsupial carnivores than in other marsupial species may be partly related to the route of infection. The primary source of *T. gondii* in carnivores is probably through the consumption of bradyzoites in tissue cysts of infected prey or carrion. While transmission of bradyzoites is the most infective form of the parasite for the cat as the definitive host (Dubey and Frenkel 1976), circumstantial evidence suggests that oocyst-transmitted infections can be more clinically severe in intermediate hosts (Bowie *et al.* 1997; Hill and Dubey 2002; Dubey 2004). This could partly explain the occurrence of clinical disease in a range of herbivore and insectivore species, including eastern barred bandicoots (*Perameles gunnii*) (Obendorf *et al.* 1996), Tasmanian pademelons (*Thylogale billardierii*) and Bennett's wallabies (*Macropus rufogriseus rufogriseus*) (Obendorf and Munday 1983), Tammar wallabies (*Macropus eugenii*) (Reddacliff *et al.* 1993), koalas (*Phascolarctos cinereus*) (Hartley *et al.* 1990) and wombats (*Vombatus ursinus*) (Skerratt *et al.* 1997), while clinical cases in the larger marsupial carnivores are notably absent. Herbivores ingest oocyst-contaminated vegetation while grazing where cats have defaecated, while bandicoots could acquire the parasite through eating soil-dwelling invertebrates that can transport oocysts mechanically on their bodies (Wallace 1971; 1972; Saitoh and Itagaki 1990). Eastern quolls consume both invertebrate and vertebrate prey (Blackhall 1980; Godsell 1983), but infection would be less severe if initial *T. gondii* infection occurred through carnivory, and the subsequent immune response could protect against subsequent exposure to oocysts. Experimental feeding trials could reveal the relative pathogenicity of different parasite sources to the eastern quoll.

The absence of clinical cases in the current study does not prove that clinical cases never occur. If eastern quolls were highly susceptible to acute toxoplasmosis, infected animals could die rapidly before serological or clinical evidence of overt disease could be observed, as occurs in eastern barred bandicoots (Bettiol *et al.* 2000). In wild populations, rapid predation of infected individuals and scavenging of carcasses would mean illness is rarely detected. In that case, reduced survival of seronegative quolls would be expected, as rapid death or predation would result in loss from the population before

seroconversion could be detected. However, we found that seronegative quolls had similar survivorship to seropositive quolls, and the high numbers of seropositive quolls in the population shows that many eastern quolls survive the initial infection. Hence, while individual occurrences may occur, the eastern quoll is unlikely to be highly susceptible to acute disease, and the high seroprevalence indicates a benign infection in this species (McCallum 1994).

The similarity in longevity of seronegative and seropositive quolls could in principle be explained by equivalent reduction in survival for both classes. While seropositive quolls survive the initial acute infection, they may then be vulnerable to predation due to risky behaviours associated with latent infection, as observed in seropositive rats (*Rattus norvegicus*) and mice (*Mus musculus*) that not only lost their fear of cats, but were attracted to them (Berdoy *et al.* 2000; Vyas *et al.* 2007). The predation of seropositive quolls may cause a reduction in survival of similar magnitude to the sudden death or predation due to acute infection that removes susceptible seronegative quolls. However, the mean longevity of 2.6 years observed in both serological categories is comparable to survival rates measured in Tasmania before the species went into decline (Godsell 1983). Accordingly, there is no evidence that a simultaneous pathogen-caused reduction in survival of both seropositive and seronegative quolls could explain the recently observed decline in quoll populations by more than 50% across Tasmania (Fancourt *et al.* 2013).

The current strain(s) of *T. gondii* at the declining sites may be more virulent than those at the non-declining site, or strains historically resident in quoll populations at the same site 20-30 years ago. Molecular epidemiological studies of *T. gondii* infections have shown significant genetic diversity, particularly in wildlife populations (Wendte *et al.* 2011; Pan *et al.* 2012; Dubey *et al.* 2013). Strains differ in their virulence and their propensity to form cysts (Carruthers and Suzuki 2007), leading to different impacts on individuals or populations (Blader and Saeij 2009). The majority of marsupial *T. gondii* infections are caused by atypical strains, with several novel alleles (Parameswaran *et al.* 2010). We did not undertake molecular identification of *T. gondii* strains, but our evidence highlights that molecular investigations should form an important part of future research into the effects of *T. gondii* infections in marsupials.

Another possibility is that the observed decline in eastern quolls may have resulted from recrudescence of latent *T. gondii* infection with environmental stressors throughout that period. The physiological effects of stress due to factors such as poor nutrition, increased predation risk and competition for food and resources, co-infection with other pathogens or the effects of habitat loss, may contribute to an increased host susceptibility and severity of infection (McCallum and Dobson 1995; Davey *et al.* 2006; Pedersen and Greives 2008). Such stressors could lead to immunosuppression of eastern quolls, thereby allowing any latent disease to recrudesce into overt clinical disease, as occurs in immunocompromised humans, including AIDS patients (Luft *et al.* 1984) and organ transplant recipients (Wendum *et al.* 2002). If this were occurring in quoll populations, for example in response to the millennium drought (2001 – 2009) (Tasmanian Planning Commission 2009), it would be evident in a reduced survival time for quolls with latent infection during this period, but not when the drought broke (2010 – 2013). However, given mean survival time for seropositive quolls was equivalent to uninfected quolls, and quoll populations have continued to decline in the post-drought period (B. Fancourt, unpubl. data), this scenario is unlikely.

Notwithstanding the apparent inability for *T. gondii* to affect eastern quoll survival, such a highly prevalent infection can often have the greatest impact on a host population through reduced fecundity (McCallum 1994). However, the mean number of pouch young produced by seropositive mothers was 50% higher than by uninfected mothers. While we were unable to assess the relative fitness of these pouch young, we found no evidence that *T. gondii* negatively affected the number of offspring produced. All the seropositive mothers captured in July came from the declining sites, so the higher reproductive output may be a function of reduced population densities and reduced competition for resources at these sites, with more nutrition available for investment in offspring. However, female body condition was actually lower at the declining sites, suggesting that the number of offspring was not driven by more favourable resource levels. Alternatively, seropositive mothers might give birth to more sons, as observed in mice (Kaňková *et al.* 2007a) and humans (Kaňková *et al.* 2007b). Such mechanisms would result in a loss of reproductive capacity as fewer females are born over successive generations. However, we were not able to test this hypothesis due to the low number of seropositive mothers captured in

July and the inability to sex pouch young at this immature stage of development. No evidence was found for *T. gondii* infection having adverse effects on male reproduction, with mean testicular volume and body condition of seropositive males both higher than those of seronegative males. Better body condition and increased testicular volume in infected males may allow them to out-compete their uninfected cohorts for mates, however, the evolutionary mechanisms driving such differences are currently not understood and warrant further investigation.

While the combined weight of evidence suggests that *T. gondii* infection is not contributing to population declines in the eastern quoll, the highly significant difference in seroprevalence between the declining sites and the non-declining site cannot be ignored. Higher seroprevalence indicates higher levels of *T. gondii* contamination in the environment at the declining sites. Under cool, moist environmental conditions, oocysts are known to be infective for at least 18 months (Yilmaz and Hopkins 1972; Frenkel *et al.* 1975). However, the similar climatic conditions at both the declining and non-declining sites suggest similar oocyst persistence in the environment at these sites. A lower number of oocysts at the non-declining site, therefore, would suggest either a lower prevalence of *T. gondii* in cats, or lower cat activity.

The high seroprevalence detected in cats across Bruny Island indicates that *T. gondii* oocysts would be prevalent in environments occupied by those cats. Therefore, the low observed prevalence in eastern quolls suggests low cat activity locally at the study site. Camera surveys confirmed that cat activity at the Bruny Island site was lower than at the declining sites. The occurrence and continued prevalence of *T. gondii* is usually dependent on the presence of cats, and prevalence is generally higher where cats are present than where they are absent (Wallace 1969; Frenkel 1974; Wallace 1976), even though transmission of cysts between intermediate hosts is possible (Tenter *et al.* 2000). The higher exposure to feral cats at the declining sites that is indicated by high prevalence of *T. gondii* infection suggests that feral cats may be contributing to suppression of quoll populations at these sites, through non-*T. gondii* related mechanisms such as predation, competition or exclusion. Future experimental manipulation of cat and quoll populations could enable evaluation of the relative impact that each of these mechanisms may have on eastern quoll populations.

3.6 Conclusion

While individual clinical cases or deaths cannot be completely ruled out, the absence of any signs of clinical toxoplasmosis in either live or dead quolls is noteworthy. When combined with the high number of seropositive individuals persisting in the population and in the absence of adverse effects on either survival or fecundity, the weight of evidence from the current study suggests that *T. gondii* infection is nonpathogenic in eastern quolls. While further research into the relative pathogenicity of different transmission modes and *T. gondii* strains is required, the eastern quoll could be considered a sentinel species for the threat of toxoplasmosis in susceptible wildlife, livestock and humans. Further research investigating the impact of feral cats on eastern quoll populations through mechanisms such as predation, competition and exclusion is needed.

Chapter 4
Regional seroprevalence of *Toxoplasma gondii* antibodies in feral and stray cats (*Felis catus*) from Tasmania

Feral cats detected as part of camera surveys at Cradoc, Tasmania.

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4.1 Abstract

Toxoplasma gondii is a cosmopolitan protozoan parasite of felids that also has significant implications for the health of wildlife, livestock and humans worldwide. In Australia, feral, stray and domestic cats (*Felis catus*) are the most important definitive host of *T. gondii* as they are the only species that can excrete the environmentally resistant oocysts that provide a major source of infection for mammals and birds. In Tasmania, the rapid decline of the Tasmanian devil (*Sarcophilus harrisii*) may allow an increase in feral cat abundance, thereby increasing the risk of *T. gondii* infection to a range of susceptible wildlife species. At present, there is scant information on the prevalence of *T. gondii* infection in feral cat populations across Tasmania. We tested feral cats from 13 regions across Tasmania for the presence of *T. gondii*-specific IgG antibodies using a modified agglutination test. Results were combined with serosurveys from three previous studies to enable a comparison of seroprevalence among 14 regions across Tasmania. We found 84.2% (224/266) of cats tested positive for *T. gondii* IgG antibodies. This is among the highest rates of prevalence recorded from Australia, and significantly higher than most other countries. Adult cats had higher seroprevalence than kittens but there was no difference between sexes. In Tasmania, seroprevalence was high in 12 of 14 regions (range: 79.3% - 100.0%), with only two regions (Tasman Island and Southern Tasmania) recording significantly lower seroprevalence (≤ 50%). This suggests a high risk of infection across Tasmania, and has significant implications for wildlife conservation should feral cat abundance increase with the ongoing declines in devils.

4.2 Introduction

Toxoplasma gondii is arguably the most significant protozoan parasite spread by felids and has a worldwide distribution (Hill *et al.* 2005; Dubey 2010). While felids are the only definitive host, infection by *T. gondii* can result in overt clinical disease (toxoplasmosis) or even death of intermediate host species (Dubey and Frenkel 1972; Innes 1997; Dubey 2010), with significant implications for public health, livestock production and wildlife conservation. Approximately one third of humans worldwide have been exposed to the parasite (Hill and Dubey 2002). Numerous species of livestock (Hartley and Marshall 1957; Munday 1970; Dubey 1986b) and wildlife (Work *et al.* 2000; Burns *et al.* 2003; Szabo *et al.*

2004) are susceptible to acute infection. Australian marsupials are particularly susceptible to toxoplasmosis (Obendorf and Munday 1983; Canfield *et al.* 1990; Innes 1997; Bettiol *et al.* 2000).

T. gondii is an intracellular coccidian parasite with a complex life cycle (Frenkel 1973). Sexual reproductive stages occur only in felids (definitive host) while asexual stages can occur in any species of mammal or bird (intermediate hosts) (Frenkel 1970; Miller *et al.* 1972; Innes 1997). Felids typically acquire the parasite through eating infected prey (Dubey and Frenkel 1976). Within 1 to 2 weeks, newly infected felids shed millions of oocysts into the environment in their faeces (Hutchison 1965; Dubey *et al.* 1970b; Frenkel *et al.* 1970; Miller *et al.* 1972; Lukešová and Literák 1998). Intermediate hosts subsequently consume infective oocysts through contaminated food, soil or water (Miller *et al.* 1972; Aramini *et al.* 1999; Hill and Dubey 2002). Once ingested, oocysts rupture and rapidly multiply as tachyzoites (Frenkel 1973), leading to clinical toxoplasmosis in some hosts. Acutely infected individuals may exhibit clinical signs including lymphadenopathy, anorexia, lethargy, incoordination, apparent blindness, disorientation, ataxia, headache, fever or death (Attwood *et al.* 1975; Obendorf and Munday 1983; 1990; Burns *et al.* 2003; Carme *et al.* 2009), although pathogenicity and symptoms vary between individuals and species. Most immunocompetent individuals, however, remain asymptomatic (Dubey *et al.* 1988; Hill and Dubey 2002), with tachyzoites differentiating into bradyzoites that form latent tissue cysts (Dubey and Frenkel 1976) predominantly in the neural and muscular tissues (Attwood *et al.* 1975; Dubey and Frenkel 1976; Canfield *et al.* 1990). Tissue cysts usually remain for the life of the host and rarely cause harm (Ekanayake *et al.* 2004; Eymann *et al.* 2006; Pusch *et al.* 2009). However, recrudescence to overt disease can occur, typically in hosts with compromised immune systems such as AIDS patients and those receiving immunosuppressive therapy for organ transplants or malignancies (Dubey 2010). Furthermore, certain risky behaviours have been associated with latent toxoplasmosis in a range of intermediate hosts including humans (Pedersen *et al.* 2011; Alvarado-Esquivel *et al.* 2013a; Galvan-Ramirez *et al.* 2013), rats (Webster *et al.* 1994; Berdoy *et al.* 2000; Vyas *et al.* 2007) and mice (Hutchison *et al.* 1980; Hay *et al.* 1983a; Hay *et al.* 1983b).

Environmental contamination with oocysts is a key factor in the transmission of *T. gondii* (Hill and Dubey 2002). In the absence of felids, some host species can transmit the parasite congenitally (Wolf *et al.* 1939; Beverley 1959; Parameswaran *et al.* 2009), sexually (Arantes *et al.* 2009; de Moraes *et al.* 2010; Santana *et al.* 2013) or through eating infected animals (Desmonts *et al.* 1965). However, in Australia, feral, stray and domestic cats (*Felis catus*) are the most significant part of the *T. gondii* life cycle as they are the only definitive host that can excrete the environmentally resistant oocysts that form a major source of infection for many intermediate hosts (Dubey *et al.* 2004). While climatic factors can strongly affect how long oocysts remain infective in the environment (Yilmaz and Hopkins 1972; Frenkel *et al.* 1975), spatial variation in both environmental contamination and disease prevalence among populations of susceptible intermediate hosts are poorly understood. At a regional scale, however, levels of primary environmental contamination are essentially a function of two key variables: local cat abundance, and prevalence of *T. gondii* infection in the local cat population.

Despite evidence that toxoplasmosis presents a significant threat to some native Tasmanian mammals (Obendorf and Munday 1983; Bettiol 2000), there is currently little reliable data for both the abundance of feral cats and the prevalence of *T. gondii* in cats in Tasmania. While some spotlight survey data is available for feral cats, spotlight surveys are known to be an unreliable method for monitoring feral cat abundance (Mahon *et al.* 1998; Molsher *et al.* 1999). Accordingly, the accuracy of cat abundance estimates and the corresponding spatial and temporal changes in abundance derived from these surveys would be questionable. Information on the spatial prevalence of *T. gondii* in Tasmanian feral cats is also limited, with published studies restricted to a few localised areas (Gregory and Munday 1976; Milstein and Goldsmid 1997; Hollings *et al.* 2013). The absence of spatially-explicit information precludes any meaningful assessment of how the risk of *T. gondii* infection may vary among populations of susceptible intermediate hosts. This information becomes increasingly important as populations of the Tasmanian devil rapidly decline as the Devil Facial Tumour Disease (DFTD) spreads across the state from east to west (Hawkins *et al.* 2006). Increased prevalence of *T. gondii* in intermediate host populations at sites where devils have already declined may be attributed to possible increases in feral cat abundance following devil decline (Hollings *et al.* 2013);

alternatively, it may simply reflect higher *T. gondii* infection prevalence in feral cat populations in those areas, or different predation rates on infected individuals in areas with different carnivore assemblages, or possibly a combination of all of these factors. But to attribute changes to any single factor, in the absence of reliable data on all of these variables, may be premature.

Cats infected with *T. gondii* typically remain asymptomatic and seroconvert soon after they have shed oocysts (Dubey and Frenkel 1972; Dubey and Thulliez 1989; Dubey *et al.* 1995a). Accordingly, for epidemiological studies, seroprevalence in feral cat populations provides an important indication of levels of environmental contamination of *T. gondii* from cats that have already shed, and therefore is a major first step in understanding regional differences in infection risk and disease prevalence.

The aim of this study was to establish the prevalence of *T. gondii* among free-ranging (feral and stray) cat populations across Tasmania. Samples were opportunistically collected from cat control activities being undertaken across the state, and were tested for the presence of *T. gondii*-specific IgG antibodies. Seroprevalence was compared among regions and to published serosurveys of cat populations from mainland Australia and other countries.

4.3 Materials and methods

4.3.1 Blood sample collection

Blood samples were collected opportunistically from feral cats captured and euthanased under cat control programs across Tasmania between 2009 and 2013. Blood was typically collected using cardiac puncture from recently killed cats. Some samples were collected from thawed carcasses that had been frozen soon after euthanasia. Whole blood samples were centrifuged for at least five minutes, with sera collected and frozen at -20°C until processed. Where known, cat location, sex and age category (independent kitten or adult) were recorded for each sample.

4.3.2 Testing for IgG antibodies

Serum samples were defrosted and tested for the presence of *T. gondii*-specific IgG antibodies using a commercial modified agglutination test (MAT) (Toxo-Screen DA, bioMérieux, France). MAT is considered to be the most sensitive test for detecting antibodies in cats (Dubey and Thulliez 1989; Dubey *et al.* 1995a) and its sensitivity and specificity have been validated (Dubey *et al.* 1995b; Dubey 1997). Haemolysis does not interfere with the test and so it can be used with serum, blood plasma or even whole blood (Dubey 2010). The announced specificity (98.8%) and sensitivity (96.2%) of the commercial kit used in this study have been validated in sera from both humans (Villard *et al.* 2012) and cats (Macrì *et al.* 2009).

Samples were treated with 2-mercaptoethanol to denature any IgM antibodies and suppress any non-specific agglutination (Desmonts and Remington 1980; Dubey and Desmonts 1987). Each serum sample was tested together with positive and negative controls supplied in the MAT kit. A positive reaction was observed when agglutination of toxoplasma formed a mat covering about half of the well base. Titres were expressed as the inverse of the highest dilution at which a positive reaction was observed. A sample was deemed positive for *T. gondii* infection if the titre was ≥ 64 (Dubey and Desmonts 1987) as this was considered conservative and consistent with similar serosurveys of feral cats on the mainland (Coman *et al.* 1981; Watson *et al.* 1982; Adams 2003; Adams *et al.* 2008). A subset of 25 samples were selected at random and retested using a second MAT kit to ensure results were consistent. All assays were tested using a blind approach such that samples or controls were not identifiable until after results were determined.

4.3.3 Data analysis

All statistical analyses were performed using R (ver. 3.0.1, R Development Core Team 2013).

4.3.3.1 Effect of age and sex

Where age and sex of the feral cat was known, seroprevalence was compared between age categories (kitten or adult), and by sex using a Fisher's Exact Test to identify any significant differences.

4.3.3.2 Regional variation within Tasmania

Samples collected and tested in the current study were grouped into 13 geographic regions, with all samples collected in close proximity (~50 km radius) pooled into a single region. Regional seroprevalence was calculated by dividing the number of positive samples as a proportion of the total number of samples collected from that region. Results from three historic studies published between 1976 and 2013 were included in the regional analysis to present a more comprehensive spatial picture using all available data, and increasing the number of regions to 14. We used a Fisher's exact test to identify any significant variation in seroprevalence among geographic regions. Due to the large number of regions, the *P*-value was calculated using a Monte Carlo simulation with 2000 replicates. To identify which regions differed, multiple pairwise Fisher's exact tests were performed and the Holm method used to adjust for any increase in type I error due to multiple comparisons (Holm 1979). Regional seroprevalence was also reviewed geographically to identify any spatial patterns.

4.3.3.3 Comparison to mainland Australia and other countries

Seroprevalence in Tasmania was compared to studies from the Australian mainland, islands and territories. As only two Australian studies used the same serologic test (MAT) as the current study, results from all available surveys were included together with details of test used and cut-off titre adopted. Seroprevalence in cats from other countries were also collated for comparison with results from Tasmania. Due to the large number of overseas studies and the known differences in sensitivity and specificity between tests (Dubey 1986b), only those serosurveys using MAT were included in statistical analyses. To enable seroprevalence to be recalculated using consistent cut-off titres (≥ 50-64), only studies that presented results for serial dilutions were included. A chi-squared test was used to identify if total seroprevalence in Tasmania differed from other Australian or overseas localities. To identify which localities differed, multiple pairwise Fisher's exact tests were performed and adjusted using the Holm method, as performed for the regional analysis.

4.4 Results

4.4.1 Effect of age and sex

Age of the cat had a significant effect on seroprevalence (odds ratio [OR] = 5.3, $P = 0.003$), with positive titres found in 88.1% (111/126) of adult cats compared to 57.9% (11/19) of kittens. There was no effect of sex, with 74.3% (52/70) seroprevalence in male cats and 81.3% (65/80) in female cats (OR = 1.0, $P = 1.000$).

4.4.2 Regional variation within Tasmania

Seroprevalence was high across 12 of the 14 regions (range: 79.3% - 100.0%) (Table 4.1). Only Tasman Island (20%) and Southern Tasmania (50%) had significantly lower seroprevalence ($P < 0.001$). No spatial pattern in seroprevalence was evident (Figure 4.1).

4.4.3 Comparison to mainland Australia and other countries

Total seroprevalence for Tasmania was 84.2% (224/266) (Table 4.1). Tasmanian seroprevalence was among the highest recorded from Australian localities (Table 4.2); only Kangaroo Island (89.4%) and Christmas Island (96.0%) had higher seroprevalence.

Seroprevalence in Tasmanian feral cats was significantly higher than in serosurveys from 28 of 35 other localities within Australia and other countries ($P < 0.001$). However, Tasmania was not significantly different to serosurveys in West Amazon, Brazil (84.1%), Addis Ababa, Ethiopia (83.3%), Majorca, Spain (83.1%), Paraná State, Brazil (77.6%), Guangzhou, People's Republic of China (76.5%), Perth, Australia (75.0%) or Mona Island, Puerto Rico (73.7%) (Table 4.3).

Table 4.1. Regional seroprevalence of T. gondii IgG antibodies from stray and feral cats in Tasmania. Results from modified agglutination test with samples deemed positive at cut-off titre ≥64, unless stated otherwise; [A] – tested using indirect, fluorescent-antibody test (IFAT) with samples deemed positive at cut-off titre ≥16; [B] – tested using latex agglutination test (LAT) with cut-off titre not specified; [C] – exact location(s) in Southern Tasmania not specified in Milstein and Goldsmid (1997); [D] – 7 of 8 samples reported as positive in Hollings *et al.* (2013), split between North west and North central regions in this study based on approximate locations (T. Hollings, pers. comm.); [E] – includes Cambridge, Mornington, Old Beach, Risdon Vale, Warrane; [F] – includes Lauderdale, Rokeby, Sandford; [G] – includes Lutana, Glenorchy, West Moonah; [H] – includes Kingston, Sandy Bay, South Hobart; NS – location not specified.

Region	Location	Samples *n*	Positive *n*	Positive %	Reference
North east	**Total**	**14**	**14**	**100.0**	
	Rushy Lagoon	9	9	100.0	This study
	Weymouth	3	3	100.0	This study
	Mt.William NP	1	1	100.0	This study
	Scottsdale	1	1	100.0	This study
North central	**Total**	**29**	**23**	**79.3**	
	Port Sorell, Low Head	22	19	86.4	This study
	Exeter	6	3	50.0	This study
	Railton area	1	1	100.0	Hollings *et al.* (2013) [D]
North west	**Total**	**8**	**7**	**87.5**	
	NS	7	6	85.7	Hollings *et al.* (2013) [D]
	Wynyard	1	1	100.0	This study
Midlands	**Total**	**56**	**54**	**96.4**	
	Campbell Town, Ross	53	[A] 51	96.2	Gregory and Munday (1976)
	Cressy	2	2	100.0	This study
	White Hills	1	1	100.0	This study
East	**Total**	**9**	**8**	**88.9**	
	Colebrook	1	1	100.0	This study
	Orielton, Sorell	4	3	75.0	This study
	Carlton River, Primrose Sands, Dodges Ferry	3	3	100.0	This study
	Fortescue Bay	1	1	100.0	This study
Hobart	**Total**	**35**	**28**	**80.0**	
	Eastern shore – north[E]	9	8	88.9	This study
	Eastern shore – south[F]	5	4	80.0	This study
	Western shore - north[G]	8	5	62.5	This study
	Western shore – south[H]	9	7	77.8	This study
	NS	4	4	100.0	This study
South east	**Total**	**11**	**11**	**100.0**	
	Judbury	6	6	100.0	This study
	Franklin	4	4	100.0	This study
	Pelverata	1	1	100.0	This study
South central	**Total**	**16**	**15**	**93.8**	
	Mt Field	5	5	100.0	This study
	Florentine	3	3	100.0	This study
	Karanja	6	6	100.0	This study
	Bushy Park	1	0	0.0	This study
	Glenfern	1	1	100.0	This study

Region	Location	Samples *n*	Positive *n*	Positive %	Reference
South	**Total** [C]	**18**	[B] **9**	**50.0**	Milstein and Goldsmid (1997)
Bruny Island	**Total**	**55**	**44**	**80.0**	This study
Tasman Island	**Total**	**5**	**1**	**20.0**	This study
Maria Island	**Total**	**2**	**2**	**100.0**	This study
Flinders Island	**Total**	**4**	**4**	**100.0**	This study
King Island	**Total**	**4**	**4**	**100.0**	This study
Tasmania	**Total**	**266**	**224**	**84.2**	

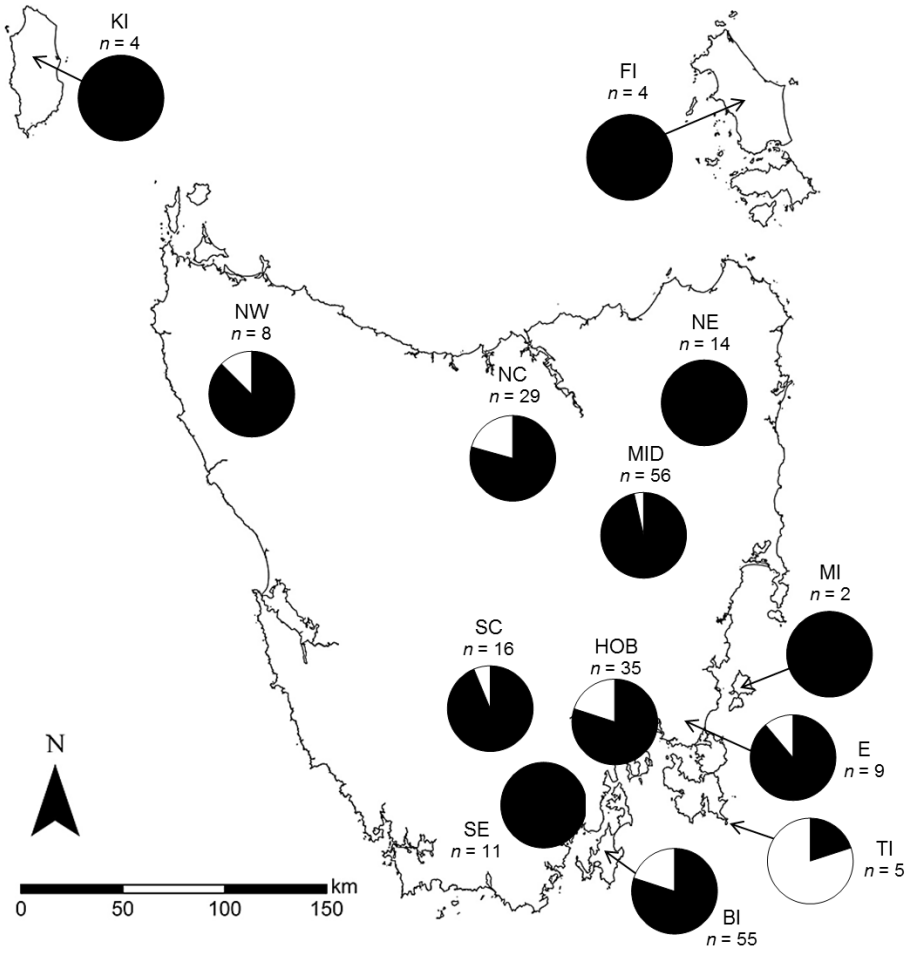

Figure 4.1. Regional seroprevalence of *T. gondii* IgG antibodies in stray and feral cats across Tasmania. Black shading indicates proportion of samples that were seropositive in each region. Regions: BI - Bruny Island, E - East, FI - Flinders Island, HOB - Hobart, KI - King Island, MI - Maria Island, MID - Midlands, NC - North central, NE - North east, NW - North west, SC - South central, SE - South east, TI - Tasman Island. Map excludes data from Milstein and Goldsmid (1997) as location only recorded as 'Southern Tasmania'.

Table 4.2. Seroprevalence of *T. gondii* IgG antibodies in stray and feral cats from the Australian mainland, territories and islands. [A] – absorbance of 0.5 at dilution of 1:1000 deemed positive titre; NS – not specified.

Location	Test	Cut-off titre	Samples *n*	Positive *n*	Positive %	Reference
Victoria, Melbourne	ELISA	[A]1000	103	40	38.8	Sumner and Ackland (1999)
Victoria, Central	IHA	64	16	7	43.8	Coman *et al.* (1981)
Victorian Mallee & riverine plains of NW Victoria & SW NSW	IHA	64	59	8	13.6	Coman *et al.* (1981)
NW WA, Shark Bay	LAT	64	42	0	0.0	Adams (2003)
WA, Perth	MAT	64	8	6	75.0	Adams (2003)
SW WA, Walpole	MAT	64	17	5	29.4	Adams (2003)
SW WA, Darling Ranges and west	IHA	NS	66	24	36.4	Jakob-Hoff and Dunsmore (1983)
SW WA, east of Darling Ranges	IHA	NS	8	0	0.0	Jakob-Hoff and Dunsmore (1983)
NSW, Sydney	IHA	64	80	42	52.5	Watson *et al.* (1982)
NSW, Sydney	NS	NS	NS	NS	30.0	Hartley cited in Hartley and Munday (1974)
SA, Kangaroo Island	DAT	4	47	42	89.4	O'Callaghan *et al.* (2005)
Christmas Island	IFAT/LAT	64	25	24	96.0	Adams *et al.* (2008)
Tasmania	MAT	64	266	224	84.2	This study (Table 4.1)

Table 4.3. Seroprevalence of *T. gondii* IgG antibodies in stray and feral cats from other countries. Table presents only comparable studies using modified agglutination test with cut-off titre ≥50-64. [A] – cut-off titre of ≥40 used; [B] – cut-off titre not specified; NS – location not specified.

Country	Location	Samples *n*	Positive *n*	Positive %	Reference
Australia	Perth	8	6	75.0	Adams (2003)
	Tasmania	266	224	84.2	This study (Table 4.1)
	Walpole	17	5	29.4	Adams (2003)
Belgium	Ghent	346	174	50.3	Dorny *et al.* (2002)
Brazil	Western Amazon	63	53	84.1	Cavalcante *et al.* (2006)
	Paraná state	58	45	77.6	Dubey *et al.* (2004)
	São Paulo	100	12	12.0	da Silva *et al.* (2002)
	São Paulo	237	73	30.8	Pena *et al.* (2006)
Colombia	NS	170	35	20.6	Dubey *et al.* (2006)
Ethiopia	Addis Ababa	48	40	83.3	Tiao *et al.* (2013)
France	Lyon	301	[A] 56	18.6	Afonso *et al.* (2006)
Grenada	NS	176	46	26.1	Dubey *et al.* (2009b)
Grenada	NS	40	7	17.5	Asthana *et al.* (2006)
Guatemala	Petén region	30	16	53.3	Lickey *et al.* (2005)
Iran	Ahvaz	100	37	37.0	Hamidinejat *et al.* (2011)
Italy	Florence	50	15	30.0	Mancianti *et al.* (2010)
	Rome	115	42	36.5	Macrì *et al.* (2009)
	Verona	490	163	33.3	D'Amore *et al.* (1997)
Mexico	Durango	150	8	5.3	Dubey *et al.* (2009d)
	Durango	105	19	18.1	Alvarado-Esquivel *et al.* (2007)
Panama	Panama City	241	[B] 110	45.6	Frenkel *et al.* (1995)
People's Republic of China	Guangzhou	34	26	76.5	Dubey *et al.* (2007b)
Portugal	Lisbon	215	[A] 44	20.5	Esteves *et al.* (2014)
	NE	204	55	27.0	Lopes *et al.* (2008)
Puerto Rico	Mona Island	19	14	73.7	Dubey *et al.* (2007a)
Spain	Barcelona	220	73	33.2	Gauss *et al.* (2003)
	Majorca	59	49	83.1	Millán *et al.* (2009)
Sri Lanka	Colombo	86	22	25.6	Kulasena *et al.* (2010)
USA	Illinois	391	234	59.8	Dubey *et al.* (1995c)
	Iowa	74	31	41.9	Smith *et al.* (1992)
	North Carolina	176	74	42.0	Nutter *et al.* (2004)
	Ohio	275	109	39.6	Dubey *et al.* (2002)
	Pennsylvania	210	37	17.6	Dubey *et al.* (2009a)
	Rhode Island	200	67	33.5	DeFeo *et al.* (2002)
West Indies	St Kitts	106	32	30.2	Moura *et al.* (2007)
	St Kitts	96	52	54.2	Dubey *et al.* (2009c)

4.5 Discussion

This study demonstrates a high seroprevalence of *T. gondii* antibodies in feral and stray cat populations throughout nearly all regions in Tasmania. Total seroprevalence in Tasmania was higher than in most other Australian localities and in nearly all other countries. This indicates a high risk of infection for a range of intermediate host species, with significant implications for wildlife conservation, livestock production and public health in Tasmania. These results are consistent with the higher infection rates evident in humans: 50-62% seroprevalence in Tasmania (Munday 1970; Milstein and Goldsmid 1997) compared to 23-35% on the Australian mainland (Garven 1957; Jennis 1963; Karunajeewa *et al.* 2001). Similar disparities have also been recorded in sheep, with 25.7% seroprevalence in Tasmania (Munday 1970) much higher than the 1.0% recorded in Queensland (Cook 1961). This high risk of infection in Tasmania is likely to increase further should feral cats, as the primary source of environmental contamination, increase in abundance following the ongoing decline of the Tasmanian devil.

4.5.1 Importance of climatic factors in environmental contamination in Tasmania

High seroprevalence in Tasmania may be attributable to favourable climatic conditions that support long-term oocyst survival in the environment. While oocysts are not infective when first shed, they sporulate and become infective after 1 to 5 days in the environment, and can remain viable for at least 18 months under certain climatic conditions (Frenkel *et al.* 1975). Oocysts persist longer in cool, moist areas than in warm, dry areas (Yilmaz and Hopkins 1972; Frenkel *et al.* 1975). Numerous studies have found correlations between climatic conditions and seroprevalence in both definitive and intermediate hosts (Coman *et al.* 1981; Almería *et al.* 2004; Afonso *et al.* 2013; Alvarado-Esquivel *et al.* 2013b). The Tasmanian climate is conducive to prolonged oocyst survival, with mean annual maximum temperatures (T) between 16 and 19°C across the state, and mean annual rainfall (P) between 500 and 1500 mm, although some parts of the central highlands receive over 2650 mm rainfall and experience colder temperatures than the rest of the state (Australian Bureau of Meteorology 2013 data). These conditions are optimal for long-term oocyst viability in the landscape, ensuring a higher level of environmental contamination than would occur in the drier, warmer areas throughout

much of the Australian mainland and in most other countries. This in turn provides an increased risk of infection to intermediate hosts and may contribute to the higher prevalence observed in Tasmanian feral cats.

Islands such as Tasmania present a closed ecosystem that may increase risk of *T. gondii* infection through a higher likelihood of exposure for intermediate hosts. The only other two Australian localities with seroprevalence greater than 80% were both islands: Kangaroo Island in South Australia and Christmas Island. While Kangaroo Island experiences similar climatic conditions (T: 19.7^{o}C, P: 491 mm) to south-eastern Tasmania, Christmas Island has both higher temperatures (T: 27.2^{o}C) and rainfall (P: 2137 mm) (Australian Bureau of Meteorology 2013 data). The high annual rainfall and relative humidity (mean annual: 82%) may in part ameliorate the desiccation effects of the higher temperatures on Christmas Island, thereby facilitating prolonged persistence. However, the closed island ecosystems of both localities restricts the geographic spread of the parasite, effectively concentrating oocyst levels on the island and increasing the likelihood of exposure for both intermediate and definitive hosts. Furthermore, island ecosystems such as Kangaroo Island and Christmas Island may support relatively higher cat densities due to the absence of an endemic top-order predator to suppress cat numbers. High seroprevalence is also evident on islands in some other countries, with Majorca and Mona Island recording among the six localities from overseas countries with the highest seroprevalence.

4.5.2 Regional variation within Tasmania

The consistently high seroprevalence in almost all regions demonstrates a high level of *T. gondii* contamination throughout Tasmania. Only two regions yielded seroprevalence below 79%. The reasons for this are unclear. The samples from southern Tasmania were tested almost 20 years earlier by Milstein and Goldsmid (1997) using a latex agglutination test (LAT), which has a different sensitivity and specificity to the MAT used in the current study. Furthermore, there was no mention of the age of cats included in the study. As the likelihood of *T. gondii* infection increases with age of the cat, a high proportion of kittens would yield a higher proportion of seronegative samples, resulting in the low observed seroprevalence for this region. To validate whether the lower seroprevalence accurately reflects a lower infection rate in this region, additional samples from cats of known age in

the region would need to be tested using MAT. However, in the absence of any specific location data, we are unable to replicate the study to validate possible reasons for the observed difference, or to investigate whether seroprevalence has increased since this study was conducted in 1997.

The 20% seroprevalence from cats on Tasman Island may reflect the recency of the parasite's introduction to the island. Cats are thought to have been first introduced to the island by lighthouse keepers in the 1940s, and it is possible that these founder cats were not carrying the parasite. As a closed island ecosystem with kittens born *in situ* being the only source of recruitment, the cat population could have maintained their *T. gondii*-free status indefinitely. However, the one cat that tested positive for IgG antibodies also tested positive for *T. gondii*-IgM antibodies (Annie Philips, Department of Primary Industries, Parks, Water and Environment (DPIPWE), unpubl. data), indicating a very recent infection in this individual, perhaps through consumption of an infected seabird that acquired the parasite elsewhere. Furthermore, as age was not recorded for these cats, samples may have come from kittens that had not yet been exposed to the parasite. The small number of samples included in the current study (n = 5) were from 2009, prior to the eradication of all feral cats from the island in 2010 (Campbell *et al.* 2011). Accordingly, we are unable to test whether seroprevalence increased on the island thereafter.

4.5.3 Implications for susceptible intermediate hosts in Tasmania

Oocyst-transmitted infections in intermediate hosts are typically more severe than tissue cyst-induced infections (Bowie *et al.* 1997; Hill and Dubey 2002; Carme *et al.* 2009). The high prevalence observed in feral cats across Tasmania suggests widespread exposure of intermediate hosts to high numbers of oocysts in the landscape. While infection in many birds and mammals may be asymptomatic and persist only as latent disease (Dubey *et al.* 1988; Canfield *et al.* 1990), toxoplasmosis is fatal to some of Tasmania's wildlife (McOrist and Smales 1986; Skerratt *et al.* 1997; Bettiol *et al.* 2000). Several other species are highly susceptible to acute disease, and may suffer a range of clinical symptoms such as ocular lesions, disorientation and ataxia that may directly or indirectly lead to increased likelihood of predation (Ashton 1979; Obendorf and Munday 1983). If the ongoing decline in devils facilitates an increase in feral cat abundance, this will result in a greater number

of oocysts being shed into the landscape and a corresponding increase in risk of disease or even death in susceptible species such as eastern barred bandicoots (*Perameles gunnii*), Tasmanian pademelons (*Thylogale billardierii*), Bennett's wallabies (*Macropus rufogriseus rufogriseus*), echidnas (*Tachyglossus aculeatus setosus*), common brushtail possums (*Trichosurus vulpecular*) and common wombats (*Vombatus ursinus*). There may also be implications for a range of other wildlife species such as Tasmanian bettongs (*Bettongia gaimardi*) and long-nosed potoroos (*Potorous tridactylus apicalis*) in which susceptibility to acute disease is currently unknown.

4.5.4 Importance of feral cats in transmission cycle in Tasmania

Feral cats are the most important part of the *T. gondii* life cycle in Tasmania and elsewhere in Australia, as they are the only resident definitive host that can excrete the environmentally resistant oocysts that provide a major source of infection for susceptible intermediate host species (Dubey *et al.* 2004). The *T. gondii* life cycle may continue indefinitely through transmission of tissue cysts between intermediate hosts (even in the absence of definitive hosts) and also by transmission of oocysts between definitive hosts (even in the absence of intermediate hosts) (Tenter *et al.* 2000). However, studies on islands, isolated human populations and properties where cats are not present have demonstrated that the continued prevalence of toxoplasmosis is usually dependent on the presence of cats, and that prevalence is generally higher where cats are present than where they are absent (Wallace 1969; Frenkel 1974; Wallace 1976). For example, Parameswaran (2008) found that marsupials located in areas where felids may roam were 14.2 times more likely to be *T. gondii* seropositive than marsupials located on felid-free islands. Even in countries with endemic wild felid species such as pumas (*Puma concolor*) and bobcats (*Lynx rufus*), domestic cats are still considered to be the major source of contamination given that oocyst formation is greatest in domestic cats (Hill and Dubey 2002).

The extent of environmental contamination from a single feral cat is considerable. Shedding is sporadic at the population level, with only around 1% of the cat population shedding at any point in time (Hill and Dubey 2002). Seronegative cats (usually kittens or young cats) typically shed within 2 weeks of first feeding on tissue cysts, and continue to shed for 1-3 weeks (Dubey *et al.* 1970a; Dubey and Frenkel 1972; Dubey 1995). However,

the millions of oocysts shed during this period (Dubey 1995) and the large home range of a feral cat of up to 10km^2 (Jones and Coman 1982b; Molsher *et al.* 2005) ensures widespread contamination of the environment in a relatively short period of time, with some cats travelling up to 45 km in 2 days (Moseby *et al.* 2009). Most infected cats shed only once in their lifetime (Dubey *et al.* 1970a; Dubey 1995). However, given that oocysts can remain viable in the environment for at least 18 months under optimal conditions (Frenkel *et al.* 1975), continuous contamination would require only a single new naïve feral cat to enter a cat population every 12 months to replenish the area with infective oocysts. Higher rates of cat recruitment, as may occur with the decline of devils, would increase the concentration of oocysts in a given area, thereby increasing the risk of infection to intermediate hosts by increasing their likelihood of exposure to oocysts.

4.5.5 Importance of intermediate hosts in transmission cycle in Tasmania

The high number of susceptible intermediate hosts in the Tasmanian ecosystem may provide a significant reservoir for the parasite and help explain the higher prevalence in Tasmanian cats in comparison to the Australian mainland and other countries. The number of intermediate hosts in a community can increase the number of reservoirs and therefore the persistence of the parasite. Cats consuming tissue cysts shed oocysts sooner and in greater numbers than cats that consume oocysts (Dubey *et al.* 1970a; Dubey and Frenkel 1976). This suggests that to maintain the high prevalence of infection observed among cat populations in Tasmania, a high availability of infected intermediate host prey species would be required. As most cats bury their faeces (Morrison 1981; Triggs 2004), species that forage by digging or grazing close to the soil have the highest likelihood of encountering and consuming oocysts. Tasmania is a refuge for Australian marsupial diversity, supporting high densities of native herbivores and ground-foraging insectivores that are known to be highly susceptible to toxoplasmosis (Johnson *et al.* 1988; Obendorf *et al.* 1996). In addition, Tasmania supports a substantial livestock industry. Sheep, cattle and pigs are all susceptible to toxoplasmosis to varying degrees (Hartley and Marshall 1957; Dubey 1986b; a). Together with a range of native and invasive small mammal species, these high numbers of susceptible intermediate hosts allow the parasite to continue its life cycle in perpetuity.

4.5.6 Future research

Reliable spatial and temporal data on feral cat abundance across Tasmania is crucial for monitoring changes in the risk of *T. gondii* infection over coming years. This study has established a high level of *T. gondii* prevalence in the Tasmanian environment. However, in order to assess any increased risk to wildlife populations between regions as devils decline, reliable data on regional cat abundance is an important next step. At present, DPIPWE record feral cat observations as part of annual spotlight surveys across most of the state (G. Hocking, DPIPWE, unpubl. data). While acknowledging that these surveys were not intended nor designed to monitor feral cats, the dataset is becoming increasingly relied upon as the only available data on the species (e.g. Hollings *et al.* 2014). However, spotlighting is not the most appropriate method for monitoring feral cats (Mahon *et al.* 1998; Molsher *et al.* 1999). While an increase in the number of cat observations may reflect a real increase in feral cat abundance over time, it may simply reflect an increase in detectability. For example, feral cats may have altered their activity both spatially and temporally in response to declining devil activity along roads that were historically frequented by devils (and possibly avoided by cats) as devils scavenged on roadkill. Such behavioural shifts by feral cats would increase their detectability in road-based spotlight surveys such as those conducted by DPIPWE (Hayward and Marlow 2014). Alternative monitoring approaches such as remote camera surveys are needed to provide a more accurate estimate of regional feral cat abundance across a range of habitats, and any temporal or spatial changes that may ensue. Reliable data on feral cat abundance in Tasmania is also critical to better inform property managers and agencies seeking to undertake cat control programs. As *T. gondii* appears to be ubiquitous throughout Tasmania, a reduction in feral cat numbers would be the most effective way to reduce levels of the parasite in the landscape and therefore reduce the risk of infection to susceptible wildlife, livestock and humans.

Chapter 5
Devil declines and catastrophic cascades: is mesopredator release of feral cats inhibiting recovery of the eastern quoll?

Tasmanian devil with advanced Devil Facial Tumour Disease at Cradle Mountain, Tasmania.

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5.1 Abstract

The eastern quoll (*Dasyurus viverrinus*) is a medium-sized Australian marsupial carnivore that has recently undergone a rapid and severe population decline over the 10 years to 2009, with no sign of recovery. This decline has been linked to a period of unfavourable weather, but subsequent improved weather conditions have not been matched by quoll recovery. A recent study suggested another mechanism: that declines in Tasmanian devil (*Sarcophilus harrisii*) populations, due to the spread of the fatal Devil Facial Tumour Disease, have released feral cats (*Felis catus*) from competitive suppression, with eastern quoll declines linked to a subsequent increase in cat sightings. Yet current evidence of intraguild suppression among devils, cats and quolls is scant and equivocal. We therefore assessed the influences of top-down effects on abundance and activity patterns among devils, feral cats and eastern quolls. Between 2011 and 2013, we monitored four carnivore populations using longitudinal trapping and camera surveys, and performed camera surveys at 12 additional sites throughout the eastern quoll's range. We did not find evidence of a negative relationship between devil and cat abundance, nor of higher cat abundance in areas where devil populations had declined the longest. Cats did not appear to avoid devils spatially; however, there was evidence of temporal separation of cat and devil activity, with reduced separation and increasing nocturnal activity observed in areas where devils had declined the longest. Cats and quolls used the same areas, and there was no evidence that cat and quoll abundances were negatively related. Temporal overlap in observed cat and quoll activity was higher in summer than in winter, but this seasonal difference was unrelated to devil declines. We suggest that cats did not cause the recent quoll decline, but that predation of juvenile quolls by cats could be inhibiting low density quoll populations from recovering their former abundance through a 'predator pit' effect following weather-induced decline. Predation intensity could increase further should cats become increasingly nocturnal in response to devil declines.

5.2 Introduction

Top predators can function as keystone species, influencing ecosystem composition and functioning through top-down processes (Paine 1980; Terborgh *et al.* 1999). Both top predators and other large predators can limit the abundance, distribution and behaviour of sympatric medium-sized predators, or 'mesopredators', which in turn could influence smaller predators, prey and plant communities (Paine 1980; Courchamp *et al.* 2003; Hayward and Slotow 2009). Top predators can suppress the abundance of mesopredators through direct killing (Palomares and Caro 1999). They can also suppress mesopredator activity by causing them to shift their spatial or temporal activity to partition limited resources or avoid aggressive interactions with larger predators (Palomares and Caro 1999; Linnell and Strand 2000; Hayward and Slotow 2009; Wang and Fisher 2012). Such shifts could lead to fitness reductions (Morris *et al.* 2009) which could in turn translate to decreased mesopredator abundance (Linnell and Strand 2000). Conversely, declining abundance of a top predator can release mesopredators from competitive pressures, allowing them to increase in abundance or adopt spatial and temporal shifts in activity that could increase their impact on competitors and prey species (Crooks and Soulé 1999; Estes *et al.* 2011; Ripple and Beschta 2012). The direction, magnitude, rapidity and duration of responses, however, are context dependant and therefore differ markedly between systems (Ripple and Beschta 2012; Allen *et al.* 2013; Nicholson *et al.* 2014; Allen *et al.* 2015; Beschta and Ripple 2015).

In Australia's island state of Tasmania (68 400 km^2), the Tasmanian devil (*Sarcophilus harrisii*; 7-11 kg) has been hypothesised to suppress smaller mesopredators such as the feral cat (*Felis catus*; 2-6 kg) (Jones *et al.* 2007), with similar size-based suppression observed in predator communities around the world (Crooks and Soulé 1999; Ritchie and Johnson 2009). The devil is the island's largest mammalian predator, following the extinction of the island's apex predator, the thylacine (*Thylacinus cynocephalus*), almost 80 years ago (Guiler 1985; McKnight 2008b). However, the species' differing feeding ecologies (Jones and Barmuta 1998; Jones and Stoddart 1998; Attard *et al.* 2011) suggests that their ecological function would also differ.

It has been suggested that the functional loss of devils from Tasmanian ecosystems could release feral cats, allowing them to increase in abundance or extend their activity to intensify predation on other species, including smaller predators such as the eastern quoll (*Dasyurus viverrinus*) (Jones *et al.* 2007). Since 1996, devil populations have undergone rapid and severe decline due to the spread of Devil Facial Tumour Disease (DFTD) (Hawkins *et al.* 2006). The largest absolute changes in devil abundance would be expected to occur in the first few years following disease arrival (McCallum *et al.* 2009). Adults have been observed to decline by around 50% per year (Lachish *et al.* 2007), and population densities reduced by 90% or more within 10 years of DFTD emergence at many sites (McCallum *et al.* 2009). These changes could vary across the landscape, due at least in part to variant forms of the disease (Hamede *et al.* 2012). At some sites (such as Cradoc and Judbury surveyed in the current study), no cases of DFTD have been recorded, despite the disease having been recorded in the region up to eight years earlier. Transmission of DFTD is strongly frequency-dependent (McCallum *et al.* 2009): even at low densities, populations have shown the same prevalence of the disease, and therefore proportionate rate of decline. However, more recent findings indicate that at extremely low densities, prevalence (and therefore rate of decline) could be reduced (Sam Fox, Save the Tasmanian Devil Program (STTDP), pers. comm.).

Evidence for a change in abundance of feral cats following devil decline is currently scant and unclear, although there are some indications that devils might be influencing cat activity. Hollings *et al.* (2014) showed an increase in feral cat sightings from spotlight surveys in NE Tasmania, coinciding with the arrival of DFTD in the region and subsequent declines in devil abundance. Suggesting that this increase in sightings reflected an increase in cat abundance, they acknowledged that behavioural shifts could also explain some of the observed increase due to changes in detectability, although it was not possible to distinguish between the two from their data set (Hollings *et al.* 2014). Contrary to their findings in NE Tasmania, the authors also observed a decrease in cat sightings following DFTD arrival in central northern Tasmania that was positively associated with sightings of native medium-sized mammals and invasive rabbits (potential prey species), indicating that responses of cats were not consistent across regions and that bottom-up processes such as food availability might also be important in driving cat

populations. More robust camera studies (i.e. of longer continuous duration, less sensitive to behavioural effects on detectability, and accounting for imperfect detection) have all found non-negative relationships between devils and cats. Saunders (2012) found a strong positive association between cat occupancy and devil abundance in DFTD-free areas supporting high devil abundance in NW Tasmania, while Troy (2014) found no numerical or behavioural relationship between devils and cats across NE, NW and southern Tasmania. Similarly, Lazenby (2012) found that feral cat population trends did not appear to be negatively affected by devils in Southern Tasmania. However, Lazenby and Dickman (2013) found that cats were detected less frequently on cameras where devils were detected, suggesting that cats might avoid areas with higher devil activity, although devils were detected more often at cameras where cats were detected. Spatial or temporal separation would minimise the likelihood of agonistic encounters (Palomares and Caro 1999) and thus indicates a reduced risk of interference competition for cats, thereby enabling them to coexist with devils. Similar separation has been observed among a number of sympatric carnivores globally (Harrington *et al.* 2009; Hayward and Slotow 2009; Ridout and Linkie 2009; Bischof *et al.* 2014).

A decline in eastern quoll abundance has been linked with increasing cat abundance or activity, inferred from an increase in feral cat sightings from spotlight surveys (Hollings *et al.* 2014). The eastern quoll is a medium-sized (0.85-2.00 kg) marsupial carnivore that has recently undergone severe and rapid decline across Tasmania (Fancourt *et al.* 2013). The species is extinct on the Australian mainland and survives only in Tasmania (Woinarski *et al.* 2014) where it has, until recently, been considered abundant and secure (McKnight 2008a). In the 10 years to 2009, the species has declined by more than 50% with no sign of recovery (Fancourt *et al.* 2013). Cats and eastern quolls have coexisted in Tasmania for over 200 years (Abbott 2008) without obvious detrimental impacts of cats on quolls; however it was suggested that, prior to the devil decline, the eastern quoll had been indirectly protected from these impacts by devils, through their suppression of feral cats (Hollings *et al.* 2014). A recent study found the prevalence of *Toxoplasma gondii* (a cat-borne parasite) was significantly higher in declined quoll populations than in a stable quoll population (Fancourt *et al.* 2014 [Chapter 3]). While *T. gondii* infection did not affect quoll survival, higher prevalence at sites where quolls had declined signalled higher feral

cat activity, implying an increased risk of cat predation and/or competition at those sites (Fancourt *et al.* 2014 [Chapter 3]). However the interactions between cats and eastern quolls have not been investigated.

Another recent study modelled the effects of climatic fluctuations on the eastern quoll's distribution and abundance, and suggested that a period of unsuitable weather (high precipitation and warm winter temperatures) had caused a rapid decline in quoll abundance between 2001 and 2003 (Fancourt *et al.* in review [Chapter 2]). However, while favourable weather conditions have since returned, quoll abundance has not recovered, suggesting that some other factor unrelated to weather is preventing recovery (Fancourt *et al.* in review [Chapter 2]). The hypothesised increase in cat abundance or activity following devil decline could explain the inability of quolls to recover.

We therefore investigated the influences of top-down effects on abundance and activity patterns among devils, feral cats and eastern quolls across the quoll's range, at sites where DFTD had first been reported in the region between 5 and 16 years earlier. We used a combination of trapping and remote camera surveys to investigate whether devils suppress cat abundance or activity, and whether cats suppress eastern quoll abundance or activity. We made four predictions: (1) feral cat abundance would be negatively related to devil abundance, and would be highest in areas where devil populations had declined the longest; (2) feral cat activity would be separated temporally and/or spatially from devil activity, and this separation would be less in areas with reduced devil activity; (3) eastern quoll abundance would be negatively related to cat abundance, and quoll abundance would be lower in areas where devil populations had declined the longest; and (4) feral cat activity would closely match eastern quoll activity in areas with reduced quoll abundance, but would differ in areas with high quoll abundance. We discuss the importance of our findings in terms of potential mesopredator release in the functional absence of a top predator, the Tasmanian devil, and the possible contribution of feral cats to the eastern quoll decline or inhibiting their recovery.

5.3 Materials and methods

5.3.1 Ethics statement

This study was carried out in accordance with the University of Tasmania Animal Ethics Committee Permit #A11655 with permission from the Tasmanian Department of Primary Industries, Parks, Water and Environment (DPIPWE) under scientific permits FA11050, FA11208, FA11295, FA12048 and FA13060.

5.3.2 Study sites

We performed longitudinal trapping and remote camera surveys at four Tasmanian study sites ('trapping sites'): Cradoc (CR), Judbury (JU), Cradle Mountain (CM) and North Bruny Island (BI) (Figure 5.1, Table 5.1). We categorised each site as 'declined' (CR, JU and CM) or 'stable' (BI) based on the population status of eastern quolls at the site. The population status for three sites (CR, CM, BI) was determined during a pilot study undertaken in 2010 (Fancourt 2010; Fancourt *et al.* 2013). The JU site was initially categorised as 'stable' based on consistent sightings from longitudinal spotlight surveys (Department of Primary Industries, Parks, Water and Environment 2011) and captures from initial trapping surveys during 2011 (this study), but was reclassified to 'declined' in early 2012 following unexpected rapid population decline. CR and JU sites were private cattle grazing properties comprising large cleared areas interspersed with intact dry sclerophyll forest. The BI site was located within a large private sheep grazing property that comprised open areas of improved pasture interspersed with remnant dry sclerophyll forest. The CM site was located in the Cradle Mountain-Lake St. Clair National Park and comprised a mosaic of cool temperate rainforest, wet eucalypt forest, mixed forest, buttongrass (*Gymnoschoerus sphaerocephalus*) moorlands and native grasslands.

We also conducted remote camera surveys at 12 additional sites across the eastern half of Tasmania ('statewide sites') (Figure 5.1, Table 5.2) within the eastern quoll's core distribution which includes Bruny Island (Fancourt *et al.* in review [Chapter 2]). Eastern quolls are predominantly associated with interfaces between forest habitat used for denning and open grasslands used for foraging (Godsell 1983). Accordingly, each survey site comprised a structural interface between forest (dry or wet eucalypt forest, mixed forest, eucalypt plantation or tall coastal scrub) and adjacent open areas (pasture or

native grasslands, buttongrass plains, harvested or immature (< 1 m height) plantation or low open coastal shrub and heathland complexes). As eastern quolls are found in a diverse range of vegetation types (Rounsevell *et al.* 1991; Taylor and Comfort 1993; Jones and Rose 1996; Fancourt *et al.* 2013), we considered vegetation structure more important than vegetation type in the current study.

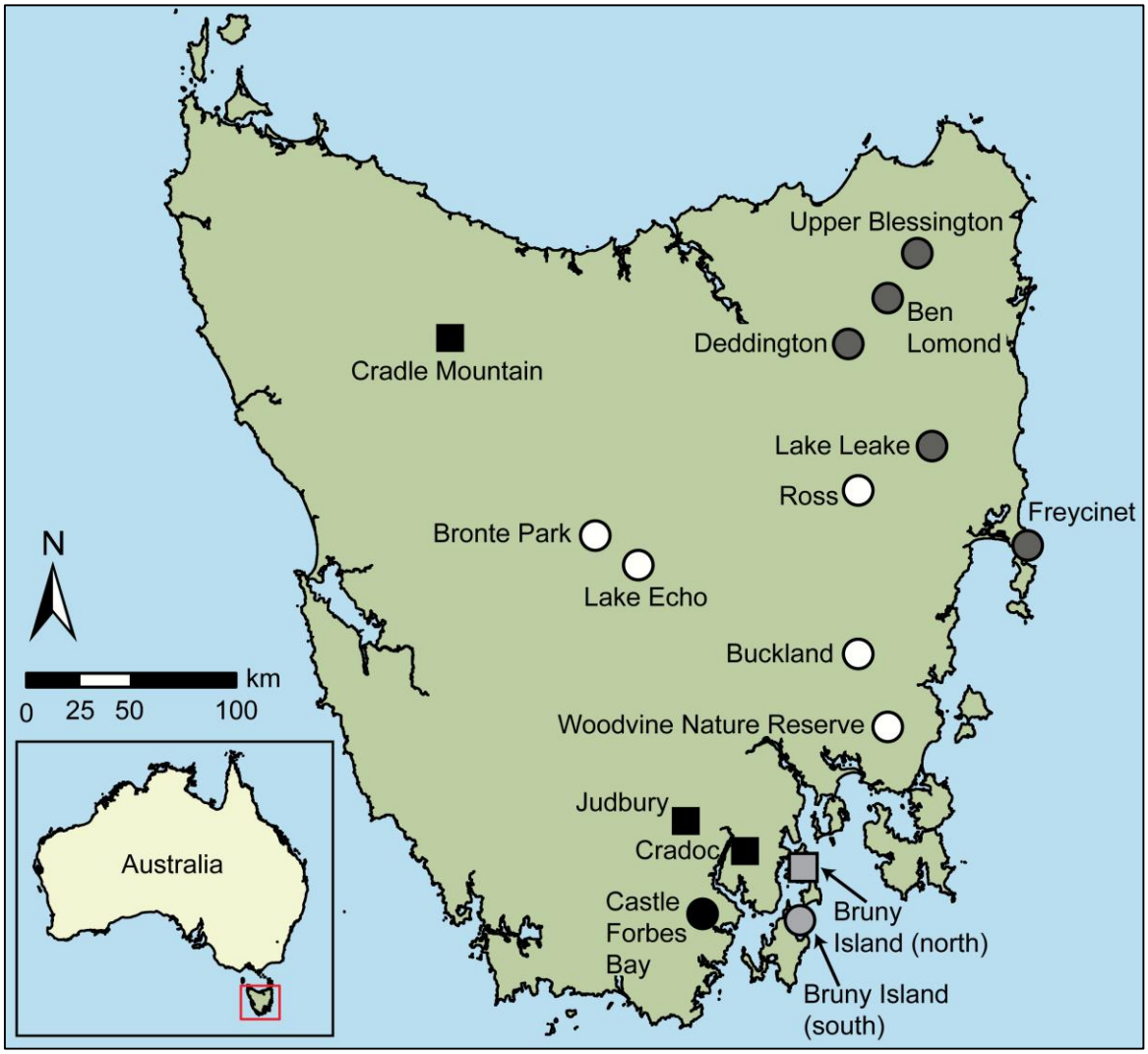

Figure 5.1. Location of study sites in Tasmania. Circles indicate sites used for statewide camera surveys, squares indicate sites used for longitudinal trapping and camera surveys. Shading indicates DFTD arrival time in region as defined in Hollings *et al.* (2014) (dark grey – early DFTD arrival (1996-1999); white – mid DFTD arrival (2000-2003); black – late DFTD arrival (2004-2007); pale grey – devil free island). Site location coordinates are listed in Tables 5.1 and 5.2. Inset shows location of Tasmania within Australia.

Table 5.1. Longitudinal population monitoring sites: locations, classifications used for data analyses and key environmental data.

[A] DFTD regions as per Hollings *et al.* (2014): E - early disease arrival (1996-1999); M - mid disease arrival (2000-2003); L - late disease arrival (2004-2007); X - devil-free island. [B] Devils: P - present; A - absent. [C] Quoll population status: CM, CR and BI sites categorised as 'declined' or 'stable', based on pilot study undertaken in 2010 (Fancourt 2010; Fancourt *et al.* 2013). JU site initially categorised as 'stable' based on consistent longitudinal spotlight surveys (Department of Primary Industries, Parks, Water and Environment 2011) and initial trapping surveys in 2011 (this study), but reclassified to 'declined' in early 2012 following rapid population decline.

Site	Site code	Location	DFTD region [A]	Devils present/ absent [B]	Quoll population status [C]	Altitude (m asl)	Mean annual precipitation (mm)
Cradle Mountain	CM	41°38'35"S, 145°57'32"E	L	P	Declined	820-950	2360
Cradoc	CR	43°06'13"S, 147°02'40"E	L	P	Declined	80-140	740
Judbury	JU	43°01'24"S, 146°54'50"E	L	P	Declined	255-275	840
North Bruny Island	BI	43°09'48"S, 147°21'17"E	X	A	Stable	30-70	670

Table 5.2. Statewide camera survey sites: locations, classifications used for data analyses and key environmental data.

[A] DFTD regions as per Hollings *et al.* (2014): E - early disease arrival (1996-1999); M - mid disease arrival (2000-2003); L - late disease arrival (2004-2007); X - devil-free island. [B] Devils: P - present; A - absent. [C] Quoll abundance: sites categorised as high or low abundance based on statistical differences in Royle Nichols abundance estimates (Supplementary material, Table S1).

Site	Site code	Location	DFTD region[A]	Devils present/ absent[B]	Quoll abundance high/low[C]	Altitude (m asl)	Mean annual precipitation (mm)
Ben Lomond	B	41°29'26"S, 147°33'16"E	E	P	High	540-640	850
Bronte Park	BP	42°04'26"S, 146°28'16"E	M	P	High	715-820	950
Buckland	BL	42°31'32"S, 147°39'03"E	M	P	Low	310-365	640
Castle Forbes Bay	CFB	43°07'23"S, 146°56'30"E	L	P	High	205-330	880
Deddington	DE	41°33'43"S, 147°26'38"E	E	P	Low	295-340	750
Freycinet	FR	42°07'35"S, 148°18'38"E	E	A	Low	10-60	690
Lake Echo	LE	42°09'38"S, 146°40'22"E	M	P	Low	865-905	810
Lake Leake	LL	41°53'25"S, 147°46'57"E	E	P	High	650-690	550
Ross	RO	42°02'05"S, 147°34'46"E	M	P	Low	250-300	490
South Bruny Island	SBI	43°18'28"S, 147°18'57"E	X	A	Low	5-30	1090
Upper Blessington	UB	41°28'38"S, 147°35'44"E	E	P	High	435-500	920
Woodvine Nature Reserve	WNR	42°47'14"S, 147°42'48"E	M	P	Low	200-250	660

5.3.3 Trapping surveys

We surveyed eastern quolls and Tasmanian devils at each trapping site using live capture and release. Any feral cats captured were removed and euthanased upon first capture. CR and JU were surveyed every second month from May 2011 to July 2012, with further surveys in January, May and July 2013. CM was surveyed every second month from May 2011 to September 2013 (except November 2012). BI was surveyed every second month from May 2011 to November 2013. We captured animals using standard PVC pipe traps baited with raw lamb heart. Traps were set within a 15 ha study area at CR, JU and BI, with traps strategically placed along the interface between the forest and adjacent open pasture. At CM, traps were set within a 200 ha study area, with traps positioned along the interface between forest and adjacent buttongrass plains or adjacent to trees or shrubs along roadsides within the open buttongrass areas. Survey effort at CR, JU and CM was 90 trap nights per survey. At BI, survey effort was usually 90 trap nights, however due to high capture rates during peak times of year, trap effort was reduced in some surveys to minimise the time quolls were kept in traps prior to processing. We marked each captured quoll or devil with an Allflex ISO-compliant FDX-B passive integrated transponder, recorded the animal's sex and age, and released the animal at the point of capture.

5.3.4 Camera surveys

We performed a three-week remote camera survey at each of the 12 statewide sites between mid-July and early November 2012. To eliminate seasonal differences between sites, we performed surveys at the time of year when quoll populations are most stable, thereby avoiding intra-annual fluctuations in eastern quoll populations that occur during the mating season (May-June) and juvenile emergence (late November-February) (Godsell 1982). The order in which sites were surveyed was designed to ensure similar sunrise and sunset times among regions; thereby ensuring region was not confounded with daylight length. For each survey, we deployed 20 RECONYX™ PC-800 passive infrared motion-detector cameras for a minimum of 21 nights. Of the three carnivore species, the eastern quoll has the smallest home range of between 35 and 44 ha (Godsell 1983). To investigate species interactions at the scale occupied by eastern quolls, we positioned cameras ca. 100 m apart along a linear 2 km transect that followed a structural interface between

open grasslands and forest. Each camera was fastened to a tree ca. 1.5 m above the ground, with a muttonbird (*Puffinus tenuirostris*) oil scent lure positioned 2–3 m in front of the camera. The camera was aimed at the ground beneath the lure, and additional muttonbird oil was drizzled on the ground in the centre of the frame. For each movement trigger, we programmed cameras to take three pictures in rapid succession, with images taken in further groups of three until movement ceased. An infrared flash was used to illuminate images at night. All images were stamped with the time, date, site and camera number. All observations of carnivore species were recorded for each survey. To minimise repeat captures of the same individual, we only treated a single detection event or 'activity' as independent if it occurred > 10 minutes after the last series of images for that species on that camera, unless individuals were distinguishable by unique pelage patterns or colours.

To corroborate trapping observations, we also conducted camera surveys at the four trapping sites. Each site was surveyed on three occasions: February/March 2012, June/July 2012 and December 2012/January 2013. Additional surveys were conducted at JU in October 2012, April/May, June and October 2013, and at CM in April, July and September 2013. For each survey, we set 20 cameras for a minimum of 21 nights using the same protocol adopted for the statewide camera surveys. However, given the key aim of these surveys, camera placement at these sites followed the transect lines used in the trapping surveys. Accordingly, these camera surveys were not directly comparable to the statewide surveys.

5.3.5 Data analysis

All statistical analyses were performed in R version 3.0.1 (R Development Core Team 2013).

5.3.5.1 Number of carnivores trapped

We compared the mean number of individual eastern quolls trapped per survey among sites using a one-factor analysis of variance (ANOVA). For this analysis, we included all survey periods from May 2011 to July 2013 but excluded data from months where surveys were not performed at all four sites during that month. Significant differences between individual sites were identified using a Tukey's pairwise comparison. We then

compared the number of quolls trapped over an annual cycle to identify any seasonal effect. For this analysis, we pooled data from the three declined quoll sites and compared the mean number of quolls trapped per survey to data from the stable quoll site for all bimonthly surveys between July 2011 and July 2012 using a two-factor repeated measures ANOVA.

We compared the mean number of devils trapped among sites using a one-factor ANOVA, and a Tukey's pairwise comparison was performed to identify which sites differed. As feral cats were only captured at the JU site and were removed when captured, we excluded cats from this analysis.

5.3.5.2 Relative abundance of carnivores

We used the camera survey data from the 12 statewide survey sites to estimate the relative abundance of eastern quolls, feral cats and Tasmanian devils at each site. For each species, we created site-specific detection histories by recording presence or absence for each camera night. We defined a camera night as the 24-hour period from 12:00:00 (midday) to 11:59:59 am on the following day. As cameras at each site were not spatially independent, we pooled detections across all 20 cameras and defined a species as 'present' on a given camera night if it was detected on at least one of the 20 cameras at that site that night. We used an occupancy modelling approach (MacKenzie *et al.* 2002) to account for the possibility that a species was present but not detected, based on the species-specific detection history for each site. To estimate relative abundance of each species, we used the Royle Nichols (RN) model (Royle and Nichols 2003) in the `unmarked` package version 0.10-3 (Fiske and Chandler 2011). The RN model is an extension of the MacKenzie *et al.* (2002) occupancy modelling approach, which recognises that variation in a species' abundance induces variation in that species' detection probability, and exploits this variation to estimate the relative abundance of the species at each site (Royle and Nichols 2003). For this analysis, we incorporated lure age (the number of days since the camera lure was deployed) as a covariate on detection probability.

We used ordinary least squares regression to determine the mean numerical relationship between devil and cat abundance across the 12 statewide camera sites. To examine

whether there was any evidence of devils imposing a limiting effect on cat abundance, we used the `quantreg` package version 5.05 (Koenker 2013) to examine the relationship between devil and cat abundance at the 50th, 75th, 95th and 99th quantiles using quantile regression. The same approach was used to investigate whether there was any evidence that cat and quoll abundance was negatively related or whether cats limit the upper abundance of quolls.

To investigate the potential for emerging trophic cascades with declining devil abundance, we also compared the abundance of devils, cats and quolls among DFTD regions. We categorised each of the statewide camera sites into early, mid or late DFTD arrival regions based on the year the disease was first reported in the region, using the same categories as Hollings *et al.* (2014) (Table 5.2). As Bruny Island is a devil-free island, we excluded the South Bruny Island (SBI) site from this analysis. We then compared the mean abundance of each species among DFTD regions using a one-factor ANOVA.

We also compared sites with high quoll abundance to sites with low quoll abundance to investigate if cat abundance was higher at sites with low quoll abundance. Sites were categorised as 'high quoll' or 'low quoll' sites based on significant differences in RN abundance estimates. Multiple pairwise comparisons were performed between sites using the `unmarked` package, with significance levels adjusted using the Bonferroni correction (α/n) to reduce the likelihood of type I error. As the Bonferroni correction could be considered too conservative for some analyses (García 2004), we corrected for alpha-inflation using $n = 11$ (for 11 comparisons between 12 sites) rather than $n = 66$ (for all 66 possible pairwise comparisons). Using this adjustment, sites separated into two distinct groups such that abundance at every 'high quoll' site was significantly higher than every 'low quoll' site. The 'high quoll' or 'low quoll' categorisation is listed for each site in Table 5.2. The relative abundance of cats and quolls was then compared between 'high quoll' and 'low quoll' sites using a one-factor ANOVA.

5.3.5.3 Spatial activity

To investigate the potential for spatial separation among carnivore species, we investigated whether cats were absent from sites where devils were present, and whether quolls were absent from sites where cats were present.

5.3.5.4 Temporal activity

To investigate the potential for temporal separation among carnivore species, we used the timestamp recorded on remote camera images to create temporal activity profiles for each species, using the `overlap` package version 0.2.3 (Meredith and Ridout 2014a). We fitted non-parametric kernel density curves using default smoothing parameters to characterise the probability density distribution of each species' activity pattern. The smoothing parameter (1/*c*) is the inverse of the concentration parameter (*c*) of the von Mises kernel (corresponding to a circular distribution) for a given sample; increasing the smoothing parameter above 1.0 produces a flatter kernel density curve while reducing it below 1.0 provides a more 'spiky' curve (Meredith and Ridout 2014b). For small sample sizes, Ridout and Linkie (2009) found that a default parameter of 0.8 minimises any over or undersmoothing of the data, thereby minimising any effect on the resulting estimators of overlap. For each species or site category pair, we then calculated the coefficient of overlapping, Δ (Weitzman 1970), as a measure of total overlap between the two species' estimated distributions. This measure ranges from 0 (no overlap) to 1 (complete overlap) and is defined as the area under the curve that is formed by taking the minimum of the two density functions at each time point. Due to the low number of cat detections in some analyses, we used the Δ_1 measure recommended for small sample sizes (Ridout and Linkie 2009) and obtained 95% confidence intervals from 10,000 smoothed bootstrap samples after accounting for bootstrap bias (Meredith and Ridout 2014b).

For each species or site category pair, we also used the non-parametric Mardia-Watson-Wheeler test in the `circular` package version 0.4-7 (Agostinelli and Lund 2013) to test for homogeneity in species activity profiles. This test detects differences in the mean angle of the circular temporal data indicative of differences in activity peaks, and requires a minimum of 10 detections for each species (Batschelet 1981). This test assumes no repeat data, so records with identical timestamps were altered by 0.001 degrees (0.24 seconds) in the raw data.

To investigate the potential for devils to affect the temporal activity of cats, we analysed activity profiles for the 11 statewide camera survey sites by DFTD region (excluding the devil-free SBI site). We also compared activity profiles of cats between sites where devils were present (*n* = 10) and those where devils were absent or undetected (*n* = 2) and also

between early and mid DFTD regions. To investigate the potential for cats to temporally suppress quoll activity, we compared activity profiles of quolls and cats at high quoll sites (n = 5) with those at low quoll sites (n = 7). To examine whether this potential changed seasonally, we compared activity profiles between cats and quolls in February, June and December 2012 at the CR site. The number of cat detections at JU, CM and BI were too low to perform a similar seasonal comparison at these sites.

5.4 Results

5.4.1 Number of carnivores trapped

We trapped significantly more individual eastern quolls per trapping survey at the stable quoll site (mean ± standard error: 30.00 ± 3.56) than at the declined quoll sites (4.85 ± 0.57) ($F_{1,2}$ = 5.62 x 10^2, P = 0.002). The number of quolls trapped at the declined sites did not differ significantly among sites (all P > 0.758).

Across the 2011-2012 annual cycle, we found a significant interaction between survey month and quoll population status ($F_{5,14}$ = 9.66, P < 0.001), with a distinct seasonal effect evident at the stable quoll site, but not at the declined quoll sites (Figure 5.2). The number of quolls trapped at the stable site in July and September increased markedly in November, and remained high until May, before decreasing again in July. We did not find any evidence of a similar marked increase at the declining sites in November, where quoll captures remained low throughout the year.

The number of quolls trapped at JU declined markedly between 2011-12 and 2012-13 (78% decline from May 2011 to May 2012; 63% decline from July 2011 to July 2012) and remained low thereafter (Figures 5.2 and 5.3(a)). Similar declines in quoll detections were observed over the seven camera surveys conducted at this site between February 2012 and October 2013 (Figure 5.3(b)). Cats were first trapped and removed from the site in May 2012 (n = 3). There were further captures and removals in July 2012 (n = 1), May 2013 (n = 1) and July 2013 (n = 1). Cats were first detected on camera in June 2012 (Figure 5.3(b)) and, despite their ongoing removal, additional detections were made in October 2012, May, June and October 2013. The number of devils captured at JU did not differ between years.

Both trapping and camera surveys detected devils at all trapping sites except BI. As expected, significantly more devils were trapped at JU than at BI where devils are absent (P = 0.018), however the number of devils trapped did not differ between other sites (all P > 0.074). Cats were not trapped at any site except JU, although they were detected on camera at all four trapping sites.

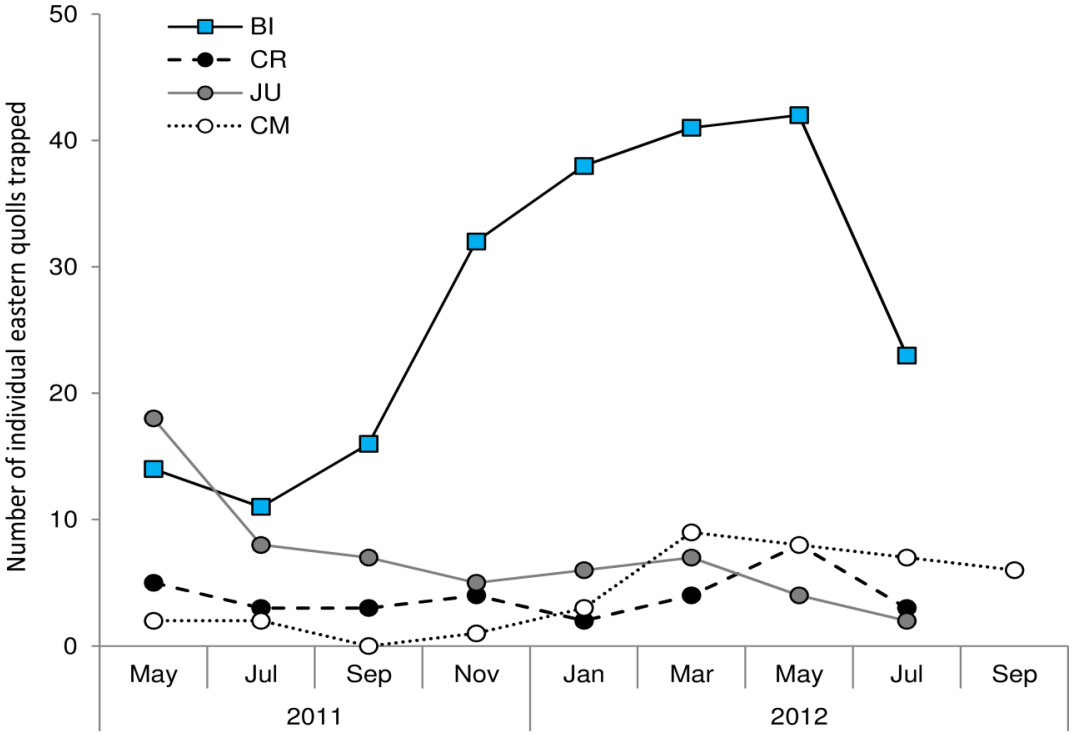

Figure 5.2. Number of individual eastern quolls captured at longitudinal trapping survey sites. Trap effort for all sites was 90 trap nights per session, except BI November 2011 (55 trap nights). North Bruny Island (BI, blue squares); Cradoc (CR, black circles); Judbury (JU, grey circles); Cradle Mountain (CM, white circles).

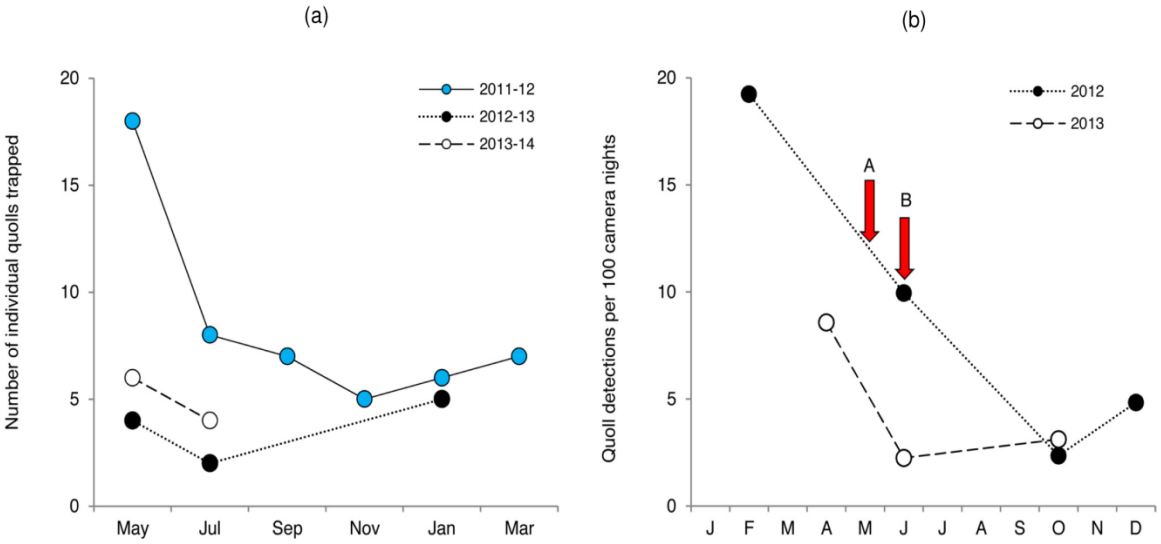

Figure 5.3. Reduction in the number of eastern quoll detections at Judbury. Plots show (a) number of individual quolls trapped per trapping survey; and (b) number of quoll detections per 100 camera nights in camera surveys. Survey effort comprised (a) 90 trap nights per survey; and (b) 20 cameras set for a minimum 21 nights. Arrows indicate the point when feral cats were first detected in trapping surveys (A) and in camera surveys (B). Trapping surveys (a) for 2011-12 were performed prior to first feral cat detection at the site; 2012-13 and 2013-14 surveys were performed after feral cats were first detected.

5.4.2 Relative abundance of carnivores

Among the statewide camera survey sites, observed cat abundance was not negatively related to devil abundance ($F_{1,10} = 1.62$, $P = 0.231$) and we did not find any evidence that devils limited the upper limit of cat abundance at any of the assessed quantiles (all $P \geq 0.145$; Figure 5.4(a)). Similarly, quoll abundance was not associated with cat abundance among the statewide camera sites ($F_{1,10} = 1.30$, $P = 0.282$) and we did not find any evidence of cats limiting the upper abundance of quolls at any of the assessed quantiles (all $P \geq 0.385$; Figure 5.4(b)). We found that while quoll abundance differed significantly between high and low quoll sites ($F_{1,10} = 29.5$, $P < 0.001$), there was no difference in cat abundance ($F_{1,10} = 1.23$, $P = 0.294$) (Figure 5.5(a)). Abundance estimates and 95% confidence intervals are listed for all species for all sites in Supplementary material, Table S2.

We did not find any evidence of trophic cascades in abundance following devil declines, with no difference in the relative abundance of quolls ($F_{2,8} = 0.29$, $P = 0.757$), cats ($F_{2,8} = 0.52$, $P = 0.611$) or devils ($F_{2,8} = 0.22$, $P = 0.805$) among DFTD regions (Figure 5.5(b)).

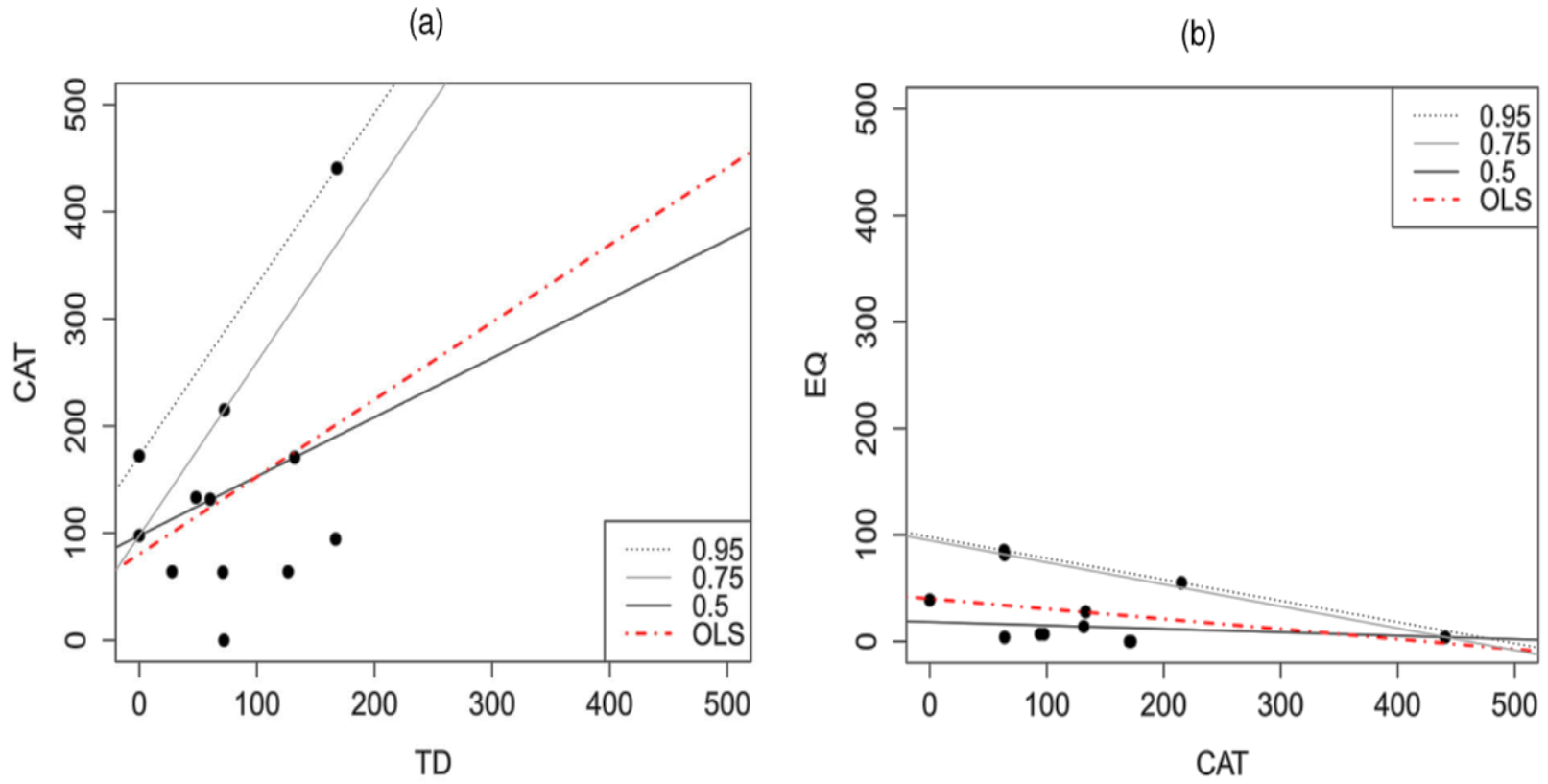

Figure 5.4. Relationship between estimated abundance of predators at statewide camera survey sites. Plots show abundance of (a) Tasmanian devils (TD) and feral cats (CAT); and (b) feral cats and eastern quolls (EQ). Each data point represents Royle Nichols abundance estimates for each species for a single camera survey site (*n* = 12 sites) as listed in Table 5.2. Regression lines shown for 50th quantile (0.5 - black, solid), 75th quantile (0.75 - grey, solid), 95th quantile (0.95 - black, dotted) and ordinary least squares (OLS - red, dot-dashed). For both figures, the lines for the 95th and 99th quantiles were identical, so only the 95th quantile line is shown.

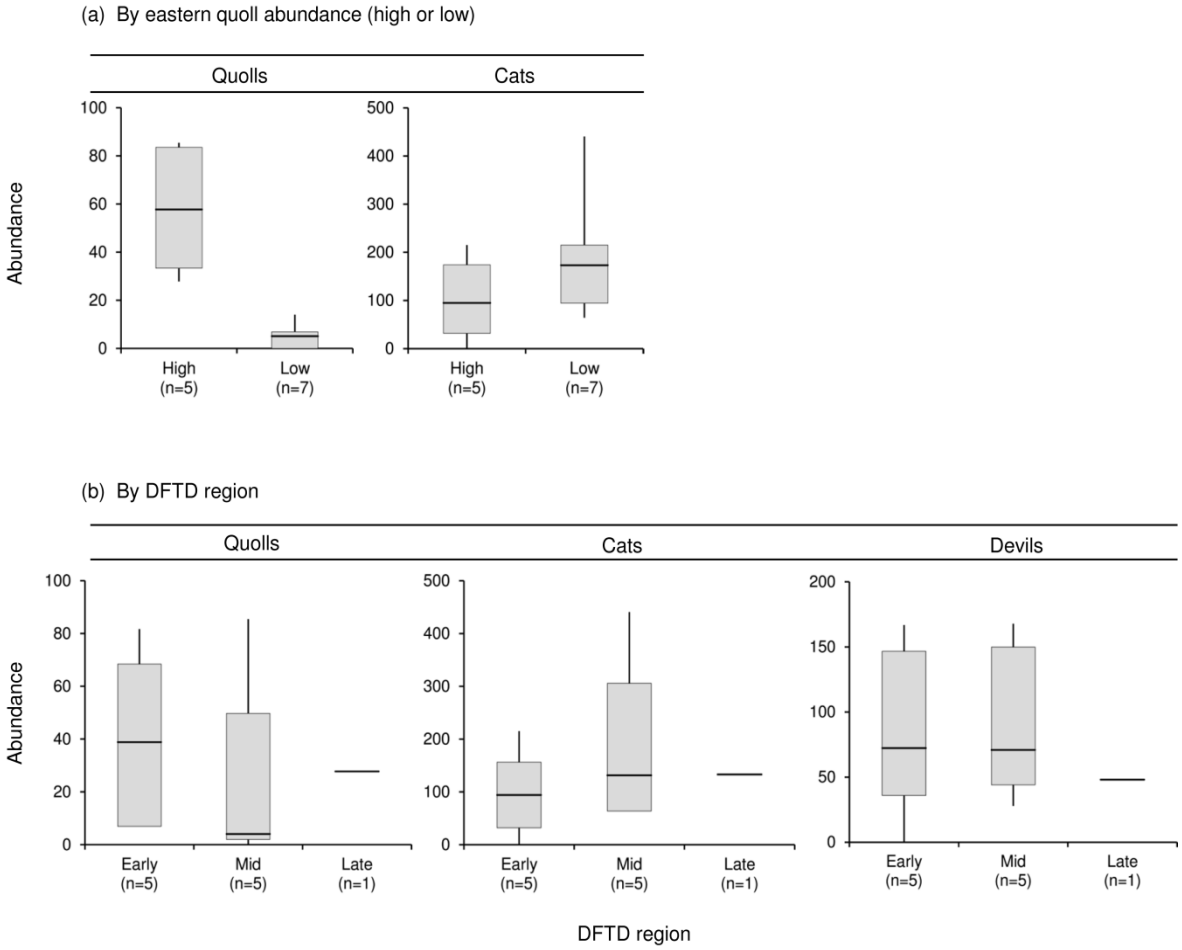

Figure 5.5. Mean abundance estimates for eastern quolls, feral cats and Tasmanian devils from statewide camera survey sites. Sites grouped by (a) high/low quoll abundance (*n* = 12 sites); and (b) DFTD arrival region (*n* = 11 sites). Sites categorised into high/low quoll abundance and DFTD regions as per Table 5.2. Analysis by DFTD region at (b) excludes data from SBI (devil-free island). Box boundaries enclose the 25th and 75th percentiles, horizontal bar is the mean, whiskers indicate maximum and minimum values. Sample sizes in parentheses indicate number of sites.

5.4.3 Spatial activity

We did not find any evidence that the presence of devils had a negative effect on local cat presence. Cats were detected at 92% (12 of 13) of camera or trapping sites where devils were detected, indicating that both species were locally active in these areas. Similarly, we did not find any evidence for local spatial separation of quolls and cats. Quolls were detected at 87% (13 of 15) of camera or trapping sites where cats were recorded.

5.4.4 Temporal activity

We found evidence of temporal separation between cats and devils (Figure 5.6(a)). Cat activity in the late DFTD region demonstrated marked separation from devil activity (Δ_1 = 0.18), although as only one site (20 cameras) was located in this region, the low number of detections precluded the calculation of confidence intervals and the performance of the Mardia-Watson-Wheeler test. Accordingly, care should be taken in further interpreting results from this region. Reduced separation was evident in the mid DFTD region (Δ_1 = 0.42 (95% CI: 0.24-0.51)), with distinct separation between peaks in cat activity (around sunset) and devil activity (peaking around midnight) (W = 43.84, P < 0.001). Separation was less evident in the early DFTD region where devils had declined the longest; total overlap in activity was higher (Δ_1 = 0.60 (0.43-0.75)), and both cat and devil activity peaked nocturnally, although peaks occurred at different times of night (W = 11.11, P = 0.004).

Differences in cat activity between early and mid DFTD regions (Δ_1 = 0.63 (0.44-0.80)) (W = 7.75, P = 0.021) (Figure 5.6(b)) were similar to differences in cat activity observed between sites with and without devils (Δ_1 = 0.62 (0.41-0.85)) (Figure 5.6(c)). Cat activity peaked around sunset in the mid DFTD region and at sites where devils were present, but peaked nocturnally in the early DFTD region and at sites where devils were absent. As there were less than 10 cat detections at sites where devils were absent, we were unable to perform the Mardia-Watson-Wheeler test for the comparison between sites with and without devils.

Quoll activity was strictly nocturnal at all statewide camera sites, however the temporal activity profiles differed between high and low quoll sites (Figure 5.7). At high quoll sites, activity peaked following sunset, and quolls remained fairly active until sunrise. At low quoll sites, the peak following sunset was notably absent, and activity peaked around midnight. Cats were active across both day and night, with a similar activity peak around sunset at both high and low quoll sites (Figure 5.7). Differences in cat and quoll activity were evident at both high (W = 6.42, P = 0.040) and low quoll sites (W = 40.20, P < 0.001). There was increased total overlap between cat and quoll activity at high quoll sites (Δ_1 = 0.62 (95% CI: 0.46-0.76)) compared to low quoll sites (Δ_1 = 0.48 (95% CI: 0.31-0.57)).

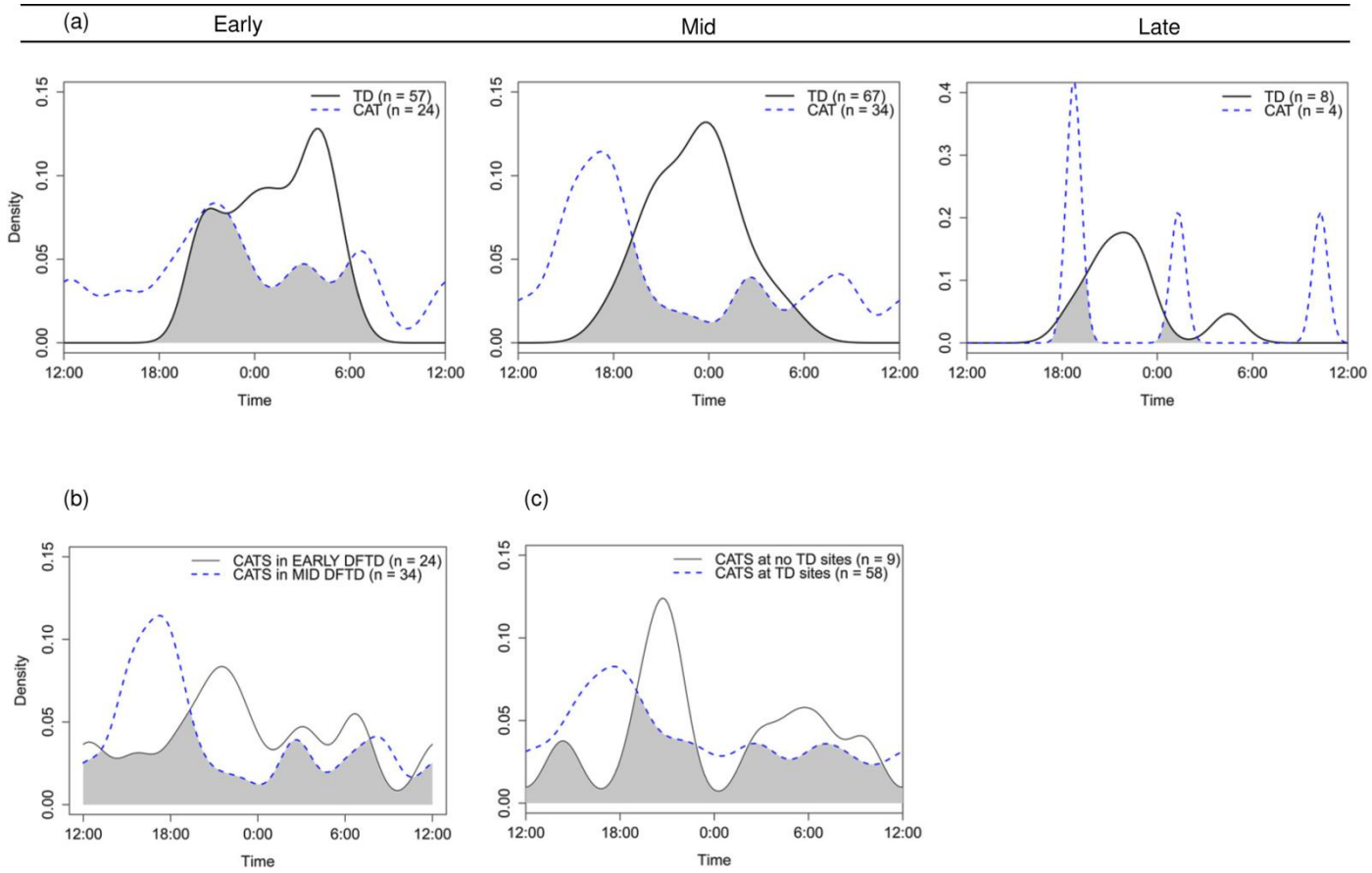

Figure 5.6. Activity of devils and cats from statewide camera survey sites. Plots at (a) show overlap of devil (TD, black solid line) and cat (CAT, blue dashed line) activity, grouped by DFTD arrival region. Sites (*n* = 11) categorised into DFTD regions as per Table 5.2 (excludes data from SBI (devil-free island)). Care should be taken in interpreting results from the late DFTD region due to the low number of detections. Plot at (b) shows difference in cat activity between early (black solid line) and mid (blue dashed line) DFTD regions (*n* = 10); and (c) shows difference in cat activity between sites with devils present (blue dashed line) and sites with devils absent (black solid line). For (c), sites (*n* = 12) categorised into devils present or absent as per Table 5.2. Sample sizes in parentheses indicate number of detection events for each species. Grey shading indicates the overlap in species' activity.

At CR, cat and quoll activity differed in February (W = 10.32, P = 0.006) and June (W = 27.56, P < 0.001) but not in December (W = 2.29, P = 0.319) (Figure 5.8). The overlap between cat and quoll activity differed seasonally (Figure 5.8). In winter, cat activity was largely crepuscular and diurnal, exhibiting minimal overlap with nocturnally active quolls (Δ_1 = 0.21 (95% CI: 0.08-0.28)). In summer, cat activity was predominantly nocturnal, resulting in increased overlap with quoll activity in both December (Δ_1 = 0.58 (95% CI: 0.37-0.80)) and February (Δ_1 = 0.51 (95% CI: 0.28-0.73)).

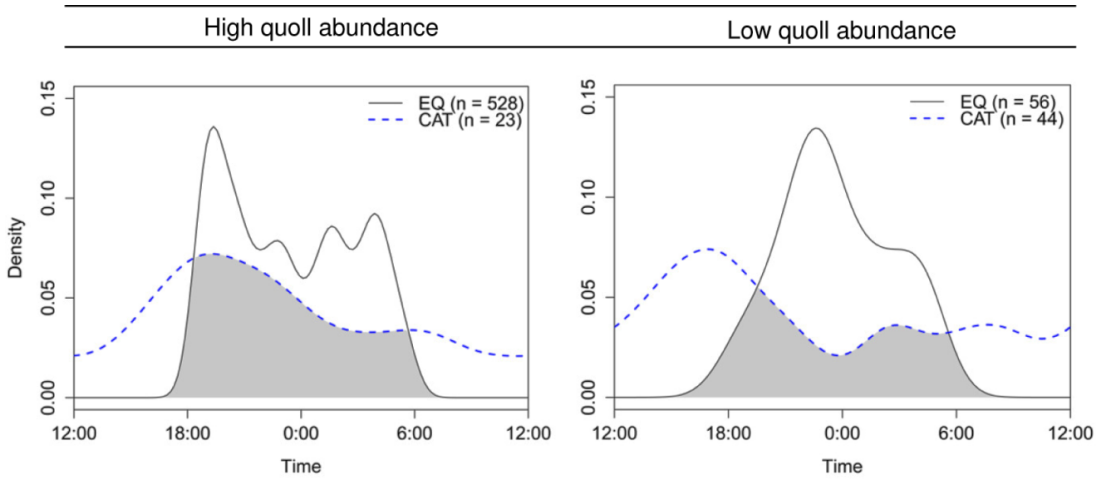

Figure 5.7. Overlap of eastern quoll and feral cat daily activity from statewide camera survey sites. Sites categorised as high (n = 5 sites) or low (n = 7) quoll abundance as per Table 5.2. Plots show overlap of quoll (EQ, black solid line) and cat (CAT, blue dashed line) activity. Sample sizes in parentheses indicate number of detection events for each species. Grey shading indicates the amount of temporal activity overlap between quolls and cats.

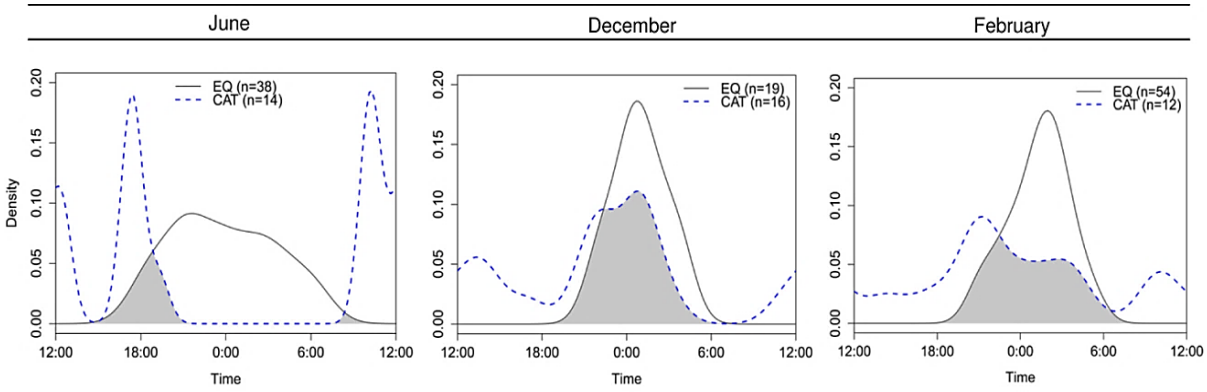

Figure 5.8. Seasonal overlap of eastern quoll and feral cat daily activity at Cradoc in 2012. Plots show overlap of quoll (EQ, black solid line) and cat (CAT, blue dashed line) activity. Sample sizes in parentheses indicate number of detection events for each species. Grey shading indicates the amount of overlap in temporal activity between quolls and cats.

5.5 Discussion

Our findings suggest that devils influence feral cat behaviour, but contrary to our prediction, we did not find any evidence that devils suppress cat abundance (Figure 5.4(a)) and there was no evidence of increased cat abundance in areas where devils had declined the longest (Figure 5.5(b)). As we predicted, observed cat and devil activity separated temporally, with separation less evident in areas where devils had declined the longest (Figure 5.6(a)). Cat activity was more nocturnal in areas where devils had declined the longest (Figure 5.6(b)). This apparent shift presents an emerging threat to nocturnal competitors and potential prey species that might have infrequently encountered cats prior to DFTD.

Contrary to our predictions, we did not find evidence to support a negative relationship between cat and quoll abundance (Figure 5.4(b)). The overlap in cat and quoll activity was greater in areas with higher quoll abundance (Figure 5.7). Overlap was also greater over summer than in winter (Figure 5.8), implying a high risk of predation for juvenile quolls. We suggest that while cats do not appear to have caused the recent quoll decline, predation of juvenile quolls by cats could be inhibiting low density quoll populations from recovering their former abundance through a 'predator pit' effect (Krebs 1996; Sinclair *et al.* 1998). Predation intensity could increase further should cats become increasingly nocturnal in response to devil declines.

5.5.1 Devil and cat interactions

Devil and cat abundance did not differ among DFTD regions (Figure 5.5(b)) and we did not find any evidence that devils suppress the abundance of cats (Figure 5.4(a)). Devil abundance did vary among sites within each DFTD region (Supplementary material, Table S2), but the similarity in mean devil abundance among regions could indicate that, below a certain density, DFTD transmission rates are reduced. This accords with findings of the Save the Tasmanian Devil Program (Sam Fox, STTDP, pers. comm.): relatively consistent, very low devil numbers with reduced disease prevalence, have been trapped in areas where DFTD has long been present. At the time of our surveys, DFTD had been present in the study region for between 5 and 16 years.

The similarity in cat abundance among regions was unexpected. There are two likely explanations. First, if devils were suppressing cat abundance prior to DFTD, the high reproductive capacity of feral cats (Jones and Coman 1982a) might have allowed rapid increase in cat abundance following the decline of devils, so that current abundance could reflect the 'post-release' abundance across regions, and the similarity in cat abundance could reflect the similarity in devil abundance among regions. If this is the case, cat abundance appears to have plateaued at new equilibrium levels across DFTD regions, with no apparent effect of time since devil decline at our survey sites (Figure 5.5(b)). While we did not find any evidence for devils suppressing or limiting cat abundance (Figure 5.4(a)), it is possible that devil densities may now be too low to be affecting cats across our survey sites, although Saunders (2012) did not find evidence of suppression at DFTD-free sites supporting high devil densities in north-west Tasmania. However, in the absence of reliable cat abundance data prior to DFTD arrival in these regions, we are unable to ascertain if current cat abundance differs from pre-DFTD abundance. An alternative explanation is that devils do not suppress cat abundance, but rather other factors, possibly bottom-up processes, could be more important in determining cat abundance, as shown by Hollings *et al.* (2014) for some regions. Different conditions promote or inhibit the transmission of predatory effects, including predator diversity, strength of interactions, ecosystem productivity, presence of refuges and the potential for compensation (Paine 1980; Pace *et al.* 1999; Oksanen and Oksanen 2000; Finke and Denno 2004; Elmhagen and Rushton 2007). For example, top-down processes might be more pronounced where there are strong productivity gradients such as in the high arctic or in arid environments, where food is limiting and competition for scarce resources is high (Elmhagen and Rushton 2007; Moseby *et al.* 2012), while predator removal in highly productive environments can result in weak effects that do not cascade through trophic levels (Chase 2003). Accordingly, Tasmania's overall higher productivity (Raupach *et al.* 2001) might promote only weak competitive interactions between devils and cats, thereby dampening any potential mesopredator release following decline of devils. Weak competitive interactions have been observed between large predators and mesopredators in other systems, such as coyotes (*Canis latrans*) and racoons (*Procyon lotor*), although the conditions necessary for these species' coexistence are not understood (Gehrt and Prange 2007). Furthermore, the prey size range and feeding

ecology of devils and cats is also quite different, with devils (carnivore/scavengers) (Jones and Barmuta 1998) unlikely to reduce or limit the availability of smaller, live prey species typically hunted by opportunistic predatory cats (Jones and Coman 1981; Denny and Dickman 2010).

The temporal partitioning of observed cat and devil activity suggests that cats could be avoiding devils. With the exception of the early DFTD region, cats were typically crepuscular or diurnal and their activity was largely separated from the nocturnally active devils (Figure 5.6(a)). In the early DFTD region where devil populations had declined the longest, cats were more nocturnal, exhibiting an increased overlap with devil activity (Figure 5.6(a)). In the absence of temporal activity data for cats and devils prior to DFTD arrival in these regions, we are unable to determine if regional differences in temporal activity are a response to declining devils, or if these differences already existed prior to DFTD arrival. However, the differences in observed cat activity between the early and mid DFTD regions (Figure 5.6(b)) are similar to the differences in observed cat activity at sites where devils were present compared with sites where devils were absent (Figure 5.6(c)). This supports the suggestion that observed differences between regions could be a response to declining devils. Further studies are needed in disease-free areas to investigate activity profiles of devils and cats prior to DFTD arrival, and to monitor if and how carnivore activity changes as DFTD spreads through the region.

The apparent response of cat activity to reduced devil abundance involves a delay, which we did not predict. A delayed response by cats could reflect the persistence of innate anti-predator responses to devils, even after selective pressures have been relaxed. For example, black-tailed deer (*Odocoilus hemionus sitkensis*) retained innate anti-predator responses to wolves (*Canis lupus*) during a ca. 100 year period of predator absence (Chamaillé-Jammes *et al.* 2014). Such behaviours could persist in the absence of a predator due to the low fitness costs associated with the behaviour (Lahti *et al.* 2009). Given the high availability of alternative abundant prey sources in Tasmania, avoidance of nocturnally active devils is unlikely to result in reduced fitness for cats. However, selective triggers, such as the drought endured in Tasmania during the three years to 2008 (Australian Bureau of Meteorology 2009; van Dijk *et al.* 2013), could have been sufficient to increase that cost due to reduced food availability, and therefore might have forced

cats to extend their hunting activities nocturnally in an effort to find limited food resources. With reduced devil abundance and reduced interference competition, nocturnal activity would now impose minimal costs to cats, enabling them (and subsequently their kittens) to specialise on nocturnal prey (Caro 1980), resulting in the gradual shift in cat activity over a few generations. Even in the absence of increasing cat abundance, temporal shifts in cat activity would present an increased predation risk for nocturnally active species such as eastern quolls that might have rarely encountered cats prior to devil decline.

Higher spotlight sightings of cats identified by Hollings *et al.* (2014) in the early DFTD region could reflect an increase in detectability rather than an increase in abundance. We did not find any evidence of higher abundance (Figure 5.5(b)), but the increased nocturnal activity of cats observed in the early DFTD region (Figures 5.6(a-b)) would likely make the cats more detectable during spotlighting surveys, which take place at night. Furthermore, while we did not find evidence of cats avoiding devils spatially in the current study, our statewide camera surveys were not performed along roads where spatial avoidance might be more evident. If devils suppress cat behaviour through interference competition, cats might have historically avoided roads where devils forage for road kills (Jones 2000), resulting in devils being detected, but cats less likely to be detected in vehicle-based spotlight surveys conducted along roads (Hayward and Marlow 2014). Following devil decline, cats might now be more active along roads and therefore more detectable in road-based spotlight surveys (Hayward and Marlow 2014). Indeed, Lazenby and Dickman (2013) found that devils can alter the detectability of cats along vehicular trails and roads, with the probability of detecting a cat often more than double at sites where devils were not detected than at sites where devils were detected. Future studies analysing GPS-movement data from sympatric devils and cats are needed to better understand the spatial interactions between these species at finer spatio-temporal scales than can be assessed using either camera or spotlight surveys.

The differing interpretations between Hollings *et al.* (2014) and this study will, in part, reflect the different collection methods and data analyses adopted. The analysis by Hollings *et al.* (2014) of statewide spotlighting data was the first study to investigate broader ecosystem effects of devil decline as they relate to a range of trophic levels, using

the best available data at that time. However, spotlight surveys are known to be an unreliable method for monitoring abundance of cryptic species such as feral cats (Mahon *et al.* 1998; Molsher *et al.* 1999). An inherent weakness of spotlight survey data is that a brief snapshot on a single night each year is likely to miss or underestimate activity that will more easily be detected by remote cameras left *in situ* for three continuous weeks. While the use of longitudinal spotlight sightings as an index of abundance does allow comparisons to be made before and after DFTD arrival, such data ignores the importance of detectability (Hayward and Marlow 2014). Accordingly, such analyses assume that the non-detection of a species means that the species was absent, whereas a non-detection could simply reflect a behaviour that makes that species less detectable in different places at different times. While longitudinal trends from spotlight surveys have been corroborated with alternative methods such as trapping surveys for devils (Hawkins *et al.* 2006) and eastern quolls (Fancourt *et al.* 2013), a similar comparison has not been performed for cats in Tasmania. Accordingly, it might be premature to presume an increase in cat sightings reflects an increase in cat abundance.

While cats appeared to avoid devils temporally, we did not find any evidence that this apparent shift in activity led to a reduction in cat abundance (Figures 5.4 and 5.6). Mammalian and avian mesopredators that avoid larger predators through temporal separation of activity can suffer reduced fitness consequences from hunting at sub-optimal times of day, with reduced resource availability and increased energy demands often leading to reduced breeding success and survival (Linnell and Strand 2000; Preisser *et al.* 2005; Sergio and Hiraldo 2008). Such costs of avoidance might be predicted to translate into reduced abundance over time. However, the similarity in cat abundance between regions with different cat activity profiles suggests that temporal shifts are not detrimental to cat fitness and abundance (Figure 5.5). Accordingly, the apparent temporal avoidance strategy adopted by cats might simply reduce their likelihood of antagonistic encounters with devils, as has been suggested with subordinate predators avoiding dominant lions (*Panthera leo*) in Africa's large predator guild (Hayward and Slotow 2009), but otherwise provides no net benefit or loss to cat abundance.

5.5.2 Interactions of cats and eastern quolls

The observed activity profiles of eastern quolls differed between sites with high and low quoll densities, but this was not related to cat activity or abundance (Figure 5.7). Temporal overlap between cats and quolls was greater at the high density quoll sites than at the low density sites, but this was a function of differing quoll activity, with no observed difference in cat activity. Given that the increased overlap was observed at higher quoll density sites, there is no indication that it has resulted in an increased predation risk to quolls. This is further supported by our finding that cat and quoll abundance were not related (Figure 5.4(b)).

The difference in quoll activity between high and low-density quoll sites could reflect differences in intraspecific competition for food. A temporal profile similar to the high density quoll sites was observed in the July 2012 camera survey on BI which supports the only confirmed stable, high density population of eastern quolls in Tasmania. The absence of devils and very low abundance of cats at this island site suggest that quoll activity is unlikely to reflect avoidance strategies in response to perceived threats from larger mammalian predators, although avian predators might still influence quoll activity. Accordingly, the similarity in the profiles between BI and the high quoll density sites on mainland Tasmania suggests that top-down processes are not a primary driver of quoll activity and that bottom-up processes are likely to be important. The delayed peak in activity around midnight at the low density sites likely reflects the reduced quoll activity in response to reduced competition for food at these sites, further supporting this hypothesis. However, to understand the influence of bottom-up processes on quoll activity, further information on the spatial and temporal variation in eastern quoll diet and activity of key prey species would be required.

The consistently low number of quolls trapped and detected at the three declined quoll sites confirms that these populations have shown no sign of recovery (Figure 5.2). Further declines were observed in both trapping and camera surveys at the JU site during the course of the study (Figure 5.3). This decline in quolls coincided with a rapid and complete decline in detections of the Tasmanian bettong (*Bettongia gaimardi*) at this site, with declines of both species coinciding with the first appearance of cats at the site (Fancourt 2014 [Appendix A]). A combination of trapping and spotlight surveys failed to detect any

cats in bimonthly surveys performed at the site between May 2011 and March 2012 or in a camera survey performed in February 2012. However, once cats were first detected in May 2012, they continued to be frequently detected on camera and regularly trapped (and removed) up to and including the final trapping survey in July 2013 and the final camera survey in October 2013 (Fancourt 2014 [Appendix A]). It is possible that cats could have been present at the site but undetected prior to May 2012, however this seems unlikely given the consistent results from a range of complementary survey techniques. While the number of quolls detected and trapped dropped rapidly, low numbers of quolls continued to be detected at the site until the end of the study. It might be that quolls at this site were initially naïve to the presence of cats, and were therefore vulnerable to predation when cats first arrived, with surviving quolls learning to avoid cats and enabling a low number of quolls to persist at this site. While these observations suggest that cats could have contributed to both quoll and bettong declines at this site, this evidence is entirely correlative and does not demonstrate causation. The decline in quolls could alternatively reflect bottom-up processes rather than top-down suppression by feral cats. However, as we did not survey prey abundance as part of the current study, we are unable to discern the mechanism(s) responsible for the quoll decline.

While we did not find any association between cats and quolls generally (Figure 5.4(b)), individual cats could have a disproportionate impact. Our statistical assessment assumes that all individuals are ecologically equivalent (Bolnick *et al.* 2003). Many populations of generalist species, such as feral cats, comprise specialised individuals whose niches are a subset of the population niche (Bolnick *et al.* 2003; Araújo *et al.* 2011). Cats are known to specialise on the type of prey with which they have had prior experience (Caro 1980) and thus individual cats can exhibit preferences in the types of prey they hunt (Dickman and Newsome in press). For example, Gibson *et al.* (1994) found that predation by two individual feral cats was catastrophic to vulnerable rufous hare-wallaby (*Lagorchestes hirsutus*) populations released into the Tanami Desert. Once these two individual cats were removed, no further killings occurred during the next 2-3 years, despite the ongoing presence of other cats in the area. Methods such as camera surveys are not appropriate to establish if and how this individual specialisation of cats might influence cat and quoll dynamics, however specialisation by individual cats provides a possible explanation for

the observed rapid decline in quolls at JU following cat incursion at this site (Figure 5.3). While predation by individual specialist cats remains one candidate agent of local decline, spatial shifts out of the local study area could also have contributed to the observed reduction in quolls at this site. Indeed, two quolls that were frequently captured prior to cat incursion were subsequently recaptured after a 12 month period of no captures following cat arrival. However, as areas surrounding the study site were not monitored in the current study, we are unable to assess the extent to which this might have occurred.

The absence of a summer spike in quoll captures at the three declined sites suggests low or no juvenile recruitment at these sites (Figure 5.2). The eastern quoll has a short, highly synchronised mating season each year, resulting in a large influx of newly independent juvenile quolls into the population between November and February each year (Godsell 1982). Numbers typically start to decline around March and usually reach pre-juvenile abundance by July each year (Godsell 1982). This characteristic annual cycle was observed at the stable site, but was notably absent at the three declined sites (Figure 5.2). Individual female quolls trapped at the declined sites had, on average, more pouch young in July (or September at CM) than quolls at the stable site (Fancourt *et al.* 2014 [Chapter 3]), indicating that individual reproductive output was not reduced. However we are unable to assess if mortality occurred while young were in dens (between August and November) or when they first emerged as independent juveniles. Demographic modelling will be required to assess whether juvenile recruitment is reduced or absent at declined sites, and whether this reflects reduced reproductive success, or mortality of newly independent or emigrating juveniles.

The high summer overlap observed between cat and quoll activity at CR (Figure 5.8) does suggest a high risk of predation to juvenile quolls, which could contribute to inadequate recruitment at the declined quoll sites. Cats are known to kill juvenile quolls (Glen *et al.* 2010). For example, two juvenile eastern quolls (360g) were killed (at different locations) from crushing injuries to the thorax and abdomen, with paired canine penetration wounds consistent with attack by a cat (B Fancourt, pers. obs). The high seasonal overlap of cat and quoll activity observed in December indicates a high predation risk to small (350-600 g) vulnerable juveniles that become independent around that time. A high degree of overlap was still evident in February when immigrating juvenile quolls are most

mobile, but had reduced by June when surviving juveniles have reached adult size. The ontogeny of decreasing vulnerability from juveniles in February to adults in May/June is reflected in the anti-predator behavioural response to cats that is exhibited by juvenile but not adult male eastern quolls (Jones *et al.* 2004). Cats might shift their activity seasonally in response to prey abundance or activity, environmental temperatures, or avoidance of larger predators. While the drivers of cat activity in this study are not known, such a seasonal shift could present a high risk to juvenile quolls in summer.

A lack of juvenile recruitment at the declined quoll sites could explain why the Tasmanian mainland populations have not recovered. As cats have been in Tasmania for over 200 years (Abbott 2008), it is highly unlikely that cat predation of juvenile quolls presents a new threat to quoll populations. Previously, the formerly high abundance of quolls might have allowed populations to sustain predation of some juveniles without having detrimental impacts on population viability. As quoll populations appear to have recently been driven to very low densities by factors unrelated to cats (Fancourt *et al.* in review [Chapter 2]), the reproductive capacity of the few persisting individuals at each site may now be insufficient to withstand the same level of predation, with declined populations now trapped in a 'predator pit' (Krebs 1996; Sinclair *et al.* 1998). Small populations are inherently more susceptible to demographic, environmental and genetic stochasticity (Shaffer 1981; Caughley 1994; O'Grady *et al.* 2004). Our findings at the high density BI site (where there have never been devils) support this hypothesis. While cats were detected during two of the three camera surveys performed at the BI site, quoll densities have remained significantly higher than at all of the declined sites, with the higher reproductive capacity of the large quoll population presumably outnumbering any losses to predation. As we did not find any evidence of cats increasing in abundance with declining devils (Figure 5.5(b)), cat predation of juvenile quolls is also unlikely to have increased following devil decline. However, the apparent delayed temporal shift in cat activity following devil decline (Figure 5.6) could further increase cat predation of eastern quolls over time.

5.5.3 Limitations and future research

We investigated interactions among devils, feral cats and eastern quolls to better understand any potential contribution to the ongoing decline and suppression of eastern quoll populations. Our study builds on the initial work and hypotheses of Hollings *et al.* (2014) by specifically examining these interactions within the eastern quoll's distribution across the drier eastern half of Tasmania. The analyses conducted by Hollings *et al.* (2014) excluded several spotlight regions in core quoll habitat in southern Tasmania and included several spotlight regions in NW Tasmania that fall outside of the core quoll distribution. Therefore, any inferences to be made regarding ecological interactions, in so far as they might be contributing to quoll declines or inhibiting quoll recovery, are limited.

Care should be taken not to over interpret our results from the late DFTD region. As most of the late DFTD region falls outside of the core eastern quoll distribution, only one of our statewide camera sites was located in the region. Our study did not investigate the potential influence of bottom-up processes such as prey activity and abundance, environmental variables and vegetation, but this should be the next logical step. However, as eastern quolls are found in almost all vegetation types excluding large tracts of rainforest (Rounsevell *et al.* 1991; Taylor and Comfort 1993; Fancourt *et al.* 2013), the increased survey effort required to achieve the necessary power to detect any differences in low-density populations may be prohibitive.

Our study is the first to investigate potential behavioural interactions among devils, cats and eastern quolls. However, as pre-DFTD data is not available to perform before-after-control-impact (BACI) analyses (Stewart-Oaten *et al.* 1986; Underwood 1992), our ability to infer whether observed differences between DFTD regions are a response to disease-induced devil declines are limited. While such BACI analyses should be performed as the disease moves through regions that are currently DFTD-free, these areas are outside the core distribution of the eastern quoll and hence any new understanding will be limited to interactions between devils and cats.

Future research should also test our hypothesis that eastern quoll populations have been reduced below a sustainable threshold from which they are unable to recover without management intervention. Even in the absence of any increase in threat following the

decline in devils, the inherent nature of small populations and their potentially ineffective population size means that natural recruitment might not be high enough to overcome established levels of threat. It may be necessary to establish insurance populations of eastern quolls, to repopulate local areas where eastern quolls have declined, with populations monitored to assess their ability to persist in the face of current, ongoing threats.

Chapter 6
General discussion

Eastern quoll pouch young, Bruny Island, Tasmania (Photo: Bronwyn Fancourt).

6.1 Overview of key thesis findings

The purpose of this study was to identify the cause of the recent decline of the eastern quoll (*Dasyurus viverrinus*) in Tasmania. By adopting a multidisciplinary approach, I was able to investigate and measure the effects of a number of candidate causal agents and determine their potential contribution to the species' decline. My investigations centred on two key events that I considered had elevated particular agents as the most likely candidate causal factors: the increasing frequency of extreme weather events such as the millennium drought, and the potential mesopredator release of feral cats (*Felis catus*) following the decline of the island's largest marsupial carnivore, the Tasmanian devil (*Sarcophilus harrisii*), due to the spread of the fatal Devil Facial Tumour Disease (DFTD).

Through the use of temporally explicit species distribution models, I provided evidence that short-term variability in weather contributed to the decline of the eastern quoll (Fancourt *et al.* in review [Chapter 2]). Recent fluctuations in the species' abundance, including a sharp decline between 2001 and 2003, were related to changes in weather across its range. However, while weather conditions improved after 2004, there was no corresponding recovery of abundance of quolls, suggesting that recovery is now being inhibited by factor(s) unrelated to weather.

I then demonstrated that despite a high susceptibility to *Toxoplasma gondii* infection, eastern quoll populations do not appear to be limited by the cat-borne parasite or its resultant disease, toxoplasmosis (Fancourt *et al.* 2014 [Chapter 3]). While *T. gondii* infection of quolls was five times higher at sites where they had declined than at the site where populations were stable, infection did not reduce quoll survival or reproduction. The prevalence of *T. gondii* in feral cats (the parasites' definitive host) did not differ among regions (Fancourt and Jackson 2014 [Chapter 4]), and therefore did not contribute to the differing prevalence of infection observed among quoll populations. However, the higher prevalence of infection in quolls at declined quoll sites did signal a higher exposure to cats at those sites (Fancourt *et al.* 2014 [Chapter 3]), lending support to the hypothesis that cats may be contributing to quoll declines and inhibiting recovery through mechanisms such as predation or competition.

Through the use of longitudinal trapping and remote camera surveys, I found that feral cats and eastern quolls used the same areas, but there was no evidence that cat and quoll abundance were negatively related (Fancourt *et al.* 2015 [Chapter 5]). While there was no difference in observed temporal activity of cats among sites with differing quoll densities, activity times of cats varied seasonally. Cat activity was typically crepuscular over winter but was more nocturnal in summer, resulting in an increased overlap with nocturnally active quolls at that time of year. Newly independent juvenile quolls emerge from their natal dens around November-December, resulting in a 3- to 4-fold increase in abundance over summer. Accordingly, such an increased overlap of cat and quoll activity at this time of year would present a high risk of predation to juvenile quolls. At sites where quolls had declined, the spike in abundance that typically accompanies juvenile emergence over summer was notably absent, suggesting that juvenile emergence is being inhibited at these sites, possibly due to cat predation of vulnerable juvenile quolls.

6.2 The cause of decline of the eastern quoll

6.2.1 A hypothesis

Based on my findings from this study, I advance a hypothesis on the cause of the recent decline of the eastern quoll in Tasmania. I suggest that a period of unsuitable weather reduced quoll populations to an unprecedented low abundance, and that populations are now too small to withstand threats to which they were robust when at higher densities. Eastern quolls appear to be trapped in a 'predator pit': environmental conditions have caused a sudden collapse in abundance, leading to a significant per capita increase in predation pressure on small surviving quoll populations, thereby preventing quolls from increasing their abundance when environmental conditions improved, and possibly contributing to further declines. Accordingly, the recent decline does not appear to be temporary and recovery is unlikely without management intervention.

The reduced abundance of eastern quolls during 2002-03 may be unprecedented in recent history, and may have taken abundance below a critical density threshold from which recovery is difficult or improbable. Throughout the 60-year modelling period (1950 to 2009), the total area of environmentally suitable habitat fell below 15,000 km^2 in only 34 months, with the 18 months from July 2002 to December 2003 representing the

longest consecutive period below 15,000 km^2. In the absence of consistent and reliable abundance records back to 1950, I cannot determine whether 2002-03 was the first instance of such low quoll abundance during this period. However, the unprecedented reduction in core habitat and the historic correlation between habitat suitability and quoll abundance suggests that the low abundance observed during 2002-03 may have also been unprecedented in this 60-year period.

The inability of eastern quoll populations to recover does not appear to have resulted from any new threat or even an increase in threat intensity, but rather an inability to overcome existing levels of threat and attain positive population growth from their current low densities. Small populations are inherently more vulnerable to demographic, environmental and genetic stochasticity (Shaffer 1981; Gilpin and Soulé 1986; O'Grady *et al.* 2004; Willi *et al.* 2006). At their former high abundance, quoll populations may have been able to withstand a certain level of mortality from predation, road mortality, non-target poisoning and a range of other pressures, without resulting in population level impacts that threaten local population persistence. However, the same threat intensities may have a disproportionately larger impact on populations that comprise fewer individuals. The loss of, say, 12 juvenile quolls to cat predation may have little or no impact on population viability or growth if the total reproductive output of the population was 100 juveniles. However, a small population with only two adult females (as was observed at many sites in the current study) can produce a maximum of 12 young per year. For these populations, the loss of 12 juveniles would remove an entire generation, thereby limiting the reproductive capacity of the population in subsequent years. Accordingly, small quoll populations may be trapped in a 'predator pit' (Kerle *et al.* 1992; Krebs 1996; Sinclair *et al.* 1998). The ability of a small population to escape from a predator pit will depend on the species' life history traits, or a reduction in predator intensity (Smith and Quin 1996). As quolls are annual breeders and can produce a maximum of 6 young per year (Godsell 1983; Bryant 1986), the species' recovery will be dependent upon management intervention, either through a reduction in predator intensity, or through supplementing quoll populations to increase local densities, or a combination of both.

6.2.2 Testing the hypothesis: an experimental approach

I recommend that an applied experimental approach be used to test my hypothesis. Field research that focuses on the manipulation of a small set of likely causal factors will provide more compelling evidence on causality than will modelling built on untested assumptions. Investigations should measure the *in situ* response of site-specific population growth rates to two distinct but possibly interacting predictor variables; eastern quoll population size and intensity of cat predation.

While the direct or indirect mechanisms responsible for the weather-induced quoll decline are not currently understood, the greatest impact on the species' viability lies in the reduced population size. As discussed above, while population size is not a cause of decline, small populations have a higher extinction risk than large populations. The inability of small populations to recover unassisted has been observed in numerous species (Newsome *et al.* 1989; Kerle *et al.* 1992; Westemeier *et al.* 1998). To quantify the effect of population size on population growth rates, I suggest that a number of sites be supplemented by the introduction of new individuals sourced from captive breeding colonies or insurance populations. The number of individuals introduced to each population should be large enough to potentially overwhelm current predation intensity (Sinclair *et al.* 1998) and facilitate a positive rate of increase under the current suite of threats. Population growth rates can then be compared between supplemented populations (large populations) and control sites where no supplementation occurs (small populations) to determine if an increased population size is sufficient to attain positive population viability.

The next hypothesis that should be tested is that the cat predation of juvenile eastern quolls is contributing to the inability of quoll populations to recover. Different species will exhibit different population responses at varying densities of predators or prey. For example, some species such as the eastern-barred bandicoot (*Perameles gunnii*) in Victoria appear to have no stable population density in the presence of exotic predators (Backhouse *et al.* 1995; Sinclair *et al.* 1998), whereas fox predation on black-footed rock wallaby (*Petrogale lateralis*) populations has a depensatory effect (inversely dependent on prey density), destabilising wallaby populations when habitat loss or weather reduce them below a threshold density (Sinclair *et al.* 1998). This latter example is a similar

scenario to the hypothesis that feral cats are suppressing recovery of quoll populations following a weather-induced decline below some critical threshold. To test whether feral cats are having a negative impact on the population growth rate of quolls, cat densities should be reduced at sites with low quoll abundance, and population changes monitored to determine whether survival of juvenile quolls improves, and if quoll populations are able to achieve a positive rate of population increase under reduced predation intensity. Sites subject to cat removal should also be compared to sites without cat removal to quantify the effect of feral cats on quoll population growth rates.

The recommended study design is presented in Figure 6.1. It comprises an eastern quoll treatment group where the size of each quoll population is increased at a number of sites through supplementation, and a quoll control group where local quoll populations at an equivalent number of sites are not supplemented. Within each group, sites should be split into two further treatment groups: a cat treatment group where feral cats are continuously removed, and a cat control group where feral cats are not removed. Quoll populations at each site should be regularly monitored before, during and after quoll supplementation and cat removal. This will enable population growth rates to be compared between treatments by using a series of planned contrasts to quantify the individual effects of each predictor, together with incremental and synergistic effects of the predictors on population viability. Temporal and spatial activity profiles of quolls and cats should also be monitored to determine any behavioural differences among treatments. The spatial scale for these 'site' manipulations should be sufficiently large enough to encompass multiple home ranges of both species, and span multiple years to ensure temporal scales are of sufficient duration to detect any population response.

The development of models of multiple causes may help to determine the relative contribution of each variable to population growth and persistence. However, the usefulness and reliability of any model output assessing population viability will depend on the accuracy and rigour of its inputs (Beissinger and Westphal 1998; McCallum 2000). In data-dependent models, uncertainties in input variables translate to uncertainties, possibly amplified, in model output. It is not always informative to postulate hypotheses, construct a model from those postulates, and then try to assess whether the real-life system under study is a realisation of this particular model; answers will only be found

after collecting and interpreting appropriate experimental raw data in the field (Pielou 1981; Beissinger and Westphal 1998; Linnell and Strand 2000). At present, we do not have quantitative data on the effects of cat predation on eastern quoll populations, nor the relationship between quoll population size and viability. Accordingly, to model the relative contribution of each of these variables based on current estimates, either as univariate or multivariate contributors, would likely produce relative meaningless predictions. Reliance on potentially inaccurate assumptions and model output may lead to misdirected and wasted management effort and potentially the loss of the species (Ferson and Burgman 1995). The most compelling evidence to support a hypothesis of cause and effect would come from longitudinal manipulative experimental testing, as I have proposed here. The findings from these experiments should then be used to develop models of multiple causes using an information-theoretic approach (Burnham and Anderson 2002), to determine the relative contribution of each factor to the population growth rate and its effect on population viability.

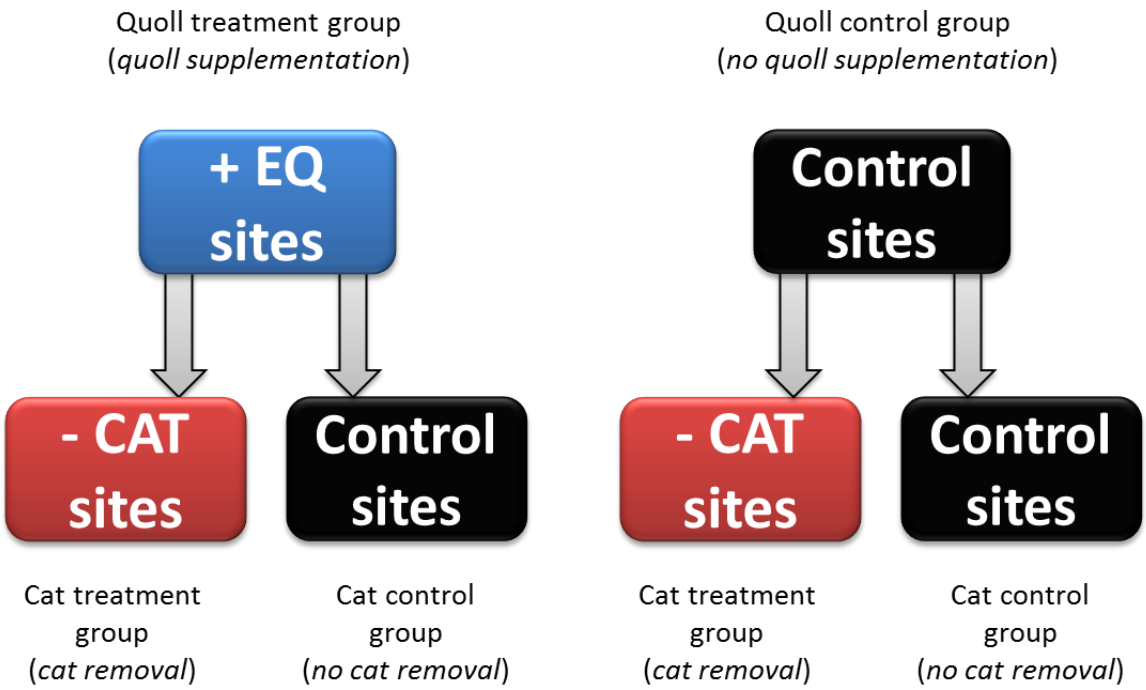

Figure 6.1. Recommended experimental design to test the hypothesis of eastern quoll decline advanced in this thesis.

6.3 Management options for conservation of the eastern quoll

The findings from this study have important implications for the future management and conservation of the eastern quoll. The study design in section 6.2.2 should be commenced as a matter of high management priority. Additionally, the following sections provide a comprehensive list of recommended management actions. Some actions will form an integral part of the recommended study design in section 6.2.2, while others are complementary and will assist in the interim conservation of the species while experimental studies are performed.

6.3.1 Do nothing

One option is not to assist the eastern quoll in recovering its former abundance, but this is not recommended. Prior to its mainland extirpation, the eastern quoll was considered widespread and sometimes overabundant throughout its range in south-eastern Australia (Peacock and Abbott 2014), illustrating that the species can rapidly descend from overabundance to extinction. While it is possible that the species may recover unassisted in Tasmania, the findings from this study suggest that this is highly unlikely. The recent loss of the Christmas Island pipistrelle (*Pipistrellus murrayi*) illustrates how inaction or delayed action can result in the extinction of a species, and that decisions must be made while there is still an opportunity to act (Martin *et al.* 2012).

6.3.2 *In situ* management

6.3.2.1 Monitoring

The importance and value of ongoing monitoring adequate to detect significant changes in eastern quoll populations cannot be overstated. The species' decline was first detected through the Tasmanian state government's annual spotlight surveys (G. Hocking, DPIPWE, unpubl. data). While these surveys were primarily established and designed to monitor wallaby and possum species subject to harvesting (Driessen and Hocking 1992), the survey method was considered valuable for monitoring long-term trends of less frequently recorded species, including the eastern quoll (Driessen and Hocking 1992). Trends in survey data were used to highlight the species' plight (WWF-Australia 2008), prompting investigations to confirm the decline (Fancourt *et al.* 2013) and identify the cause(s) of decline (this study) within a reasonable timeframe. Long-term trends from

these spotlight surveys have now been confirmed for the eastern quoll using trapping surveys (Fancourt *et al.* 2013) and remote camera surveys (Fancourt *et al.* 2015 [Chapter 5]). In the absence of alternative monitoring protocols for the species, spotlight surveys should continue as an interim form of monitoring. However, given the parlous status of the species, more robust monitoring techniques such as trapping and remote camera surveys are warranted to ensure that conservation of the species is adaptive. Trapping surveys enable collection of demographic data and biological samples, but are labour intensive and restricted in their spatial coverage. However, remote camera surveys are non-invasive, relatively inexpensive, less labour-intensive than trapping surveys, and do not require proximity to roads. They provide more extensive data sets than vehicle-based spotlight surveys conducted once a year along roads. Importantly, camera surveys enable detection probability to be incorporated into any estimates of species occupancy or abundance, and facilitate assessments of behavioural responses such as spatial and temporal activity patterns that are not discernible using techniques such as spotlight or trapping surveys.

Only two relatively high-density populations were confirmed in this study: North Bruny Island and Upper Blessington, although the latter site supported a much lower density than North Bruny Island. A third high-density site was initially confirmed at Bronte Park, however a repeat survey 12 months later confirmed an 80% reduction in eastern quoll detections in comparison to the first survey. While the island population on North Bruny Island is isolated from many of the pressures currently threatening populations on mainland Tasmania, its isolation also renders it extremely vulnerable to catastrophic events such as bushfires or the introduction of a novel disease. This population is already at risk of inbreeding depression (Hedrick and Kalinowski 2000) due to its low genetic diversity (Cardoso *et al.* 2014), further compounding the vulnerability to threats such as infectious disease. Monitoring and active management of these two populations is critical to the conservation of the species in the wild. Management actions should focus on retaining or increasing genetic variation for each of these key populations, and ameliorating threatening processes at each site. The small and overlapping home range of the species makes these actions more feasible. Future research should also focus on identifying the characteristics of these two populations to understand why they haven't

declined, or why they have recovered from decline when other populations have not. Remote camera surveys should also be extended to sites not surveyed as part of the current study, to potentially identify any other surviving high density quoll populations that warrant intensive management.

6.3.2.2 Feral cat control

The regular removal of feral cats from sites supporting low densities of eastern quolls may allow quolls to emerge from the 'predator pit' and recover their former abundance (Fancourt *et al.* 2015 [Chapter 5]). The numerical reduction of feral cats could reduce the likelihood of a quoll encountering a cat, thereby reducing the risk of predation. Additionally, if certain individual cats specialise on quolls as prey (Caro 1980; Dickman and Newsome in press), their removal could have a disproportionately positive impact on quoll populations. However, the converse may also apply if a single quoll specialist cat remains after all other cats are removed, as a large numerical reduction in cats would only result in a minimal reduction in predation risk. Limited management resources should concentrate removal efforts in October-November each year, thereby reducing predation intensity over summer when vulnerable juvenile quolls first emerge from their natal dens.

The total eradication of cats is not a realistic objective in an area as large as Tasmania, but control programs should aim at reducing cat abundance in priority areas when and where species of sensitive prey are most vulnerable. While the total removal of predators from islands can be achieved, successful outcomes typically require large investments of resources (Courchamp *et al.* 2003; Nogales *et al.* 2004; Campbell *et al.* 2011; Robinson and Copson 2014). Such efforts are unlikely to be economically and logistically feasible in large areas of continuous landscapes. Furthermore, targeted cat removal programs in open populations can sometimes result in temporary localised increases in cats due to reinvasion from surrounding areas (Lazenby 2012; Stobo-Wilson 2014). Accordingly, sustainable ecosystems need to be managed in the presence of predators, possibly by reducing abundance so that species can develop appropriate anti-predator responses, such as spatial or temporal partitioning of resources, thereby adapting to live sympatrically with their predators (Lima and Dill 1990; Creel *et al.* 2005). Coexistence is a prerequisite for biodiversity persistence (Linnell and Strand 2000). But for some species in

some ecosystems, coexistence may not be possible, as may be the case for eastern barred bandicoots and cats in Victoria (Backhouse *et al.* 1995; Sinclair *et al.* 1998).

6.3.2.3 Devil declines

Compounding the threats posed by feral cats are the shifting ecosystem dynamics following the decline of the devil due to the spread of DFTD. The functional loss of devils from Tasmanian ecosystems could release feral cats, allowing them to increase in abundance or extend their activity to intensify predation on other species, including smaller predators such as the eastern quoll (Jones *et al.* 2007). While an increase in cat sightings in the north-east of the state has been linked to declining devil abundance following DFTD arrival (Jones *et al.* 2007; Hollings *et al.* 2014), there is currently no evidence supporting the hypothesis that devil and cat abundance are negatively related, or that cat abundance has increased following devil decline (Lazenby 2012; Saunders 2012; Troy 2014; Fancourt *et al.* 2015 [Chapter 5]). However, the mechanisms by which devils could suppress cats may be more subtle, with some evidence supporting the hypothesis that cats may avoid devils temporally (Lazenby and Dickman 2013; Fancourt *et al.* 2015 [Chapter 5]). Differences in cat activity with increasing time since DFTD arrival suggest that cats may be becoming more nocturnal as devils decline, with similar differences observed among sites with and without devils (Fancourt *et al.* 2015 [Chapter 5]). If this is the case, then shifting cat activity presents an emerging threat to nocturnal species such as eastern quolls that may have rarely encountered cats before devil decline. In this way, predation risk from feral cats may increase further as devils continue to decline, even without an increase in cat abundance. The monitoring of devil and cat populations before, during and after DFTD arrival in the disease-free areas of western Tasmania would help clarify whether devils at higher densities can suppress cats numerically, and if temporal differences observed in the current study are a response to devil decline or merely reflect pre-existing differences between regions due to other factors that may differ regionally. If devils do suppress cat activity, restoration of devil populations may help ameliorate the predation intensity on nocturnal species such as the eastern quoll.

6.3.2.4 Other local threatening processes

Ongoing efforts to eradicate the introduced red fox (*Vulpes vulpes*) should continue as a high management priority in Tasmania. As there has been no confirmed fox evidence in Tasmania since July 2011 (Invasive Species Branch 2013), the Fox Eradication Plan is currently in its final stage of operations, with a focus on statewide monitoring and incursion response (Department of Primary Industries, Parks, Water and Environment 2014a). Should foxes become established, the increased predation intensity would not only threaten current low density eastern quoll populations with extinction, but would likely result in the widespread decline of critical weight range species, as seen on the Australian mainland (Woinarski *et al.* 2014).

While not specifically addressed in the current study, other potential threats identified in Chapter 1, such as habitat loss and non-target poisoning from rodenticides, should also be investigated to better understand their impact on eastern quoll populations. I did not consider these factors to be highly likely candidate causal agents in the recent decline, however they may act in combination with other factors to produce synergies that may amplify negative impacts (Brook *et al.* 2008), particularly on current small quoll populations. Dietary studies should be undertaken to understand the extent to which feral cats compete with eastern quolls for resources. Demographic modelling should also be performed to identify which key life stages appear to differ between declined and stable quoll populations, thereby revealing the causal agents' mode of action.

6.3.2.5 Climate change

The decline in eastern quoll abundance appears to be linked to an unusual period of unsuitable weather, and the frequency, severity and duration of extreme weather events are predicted to increase over coming decades as a result of anthropogenic-driven climate change (White *et al.* 2010; IPCC 2013). The predicted increase in minimum winter temperatures and increased frequency and intensity of extreme rainfall events will gradually erode environmental suitability for eastern quolls. Furthermore, the increasing frequency of these unfavourable events will increase the frequency with which populations will be reduced. If quoll populations are unable to recover unassisted under current threat intensities, subsequent extreme weather events may compound the problem and drive current small populations to extinction.

While this study identified which weather variables are important to the likelihood of quoll occurrence (i.e. the minimum temperature of coldest month and precipitation of the wettest quarter), further investigation is required to understand how these variables affect eastern quolls, including how they may interact with other threats. For example, do minimum winter temperatures affect seasonal breeding cues, or determine food resources? An understanding of these mechanisms will help managers decide on actions to ameliorate impacts on quoll populations. The broader causes of climate change are driven by global processes and therefore cannot be adequately managed at the local population scale. Management should focus on reducing the intensity of current threats such as feral cats, non-target poisoning, habitat loss and road mortality, to increase the likelihood of quoll recovery following weather-induced declines in abundance.

6.3.3 *Ex situ* management

6.3.3.1 Insurance populations

The establishment of insurance populations should be considered a high management priority. Captive populations would serve two main purposes: to provide individuals to supplement current low-density populations in Tasmania, and to numerically and genetically insure against the loss of the species in the wild. The species readily adapts to captive management and breeds well in captivity (Bryant 1988). However, the use of large fenced predator-free reserves (e.g. the Mt Rothwell Conservation and Research Centre in Victoria and the Secret Creek Sanctuary near Lithgow in NSW) facilitates the conservation of wild-living, self-sustaining eastern quoll populations while minimising their dependence on humans, thereby allowing quolls to better retain their natural instincts and behaviours. These free-range enclosures, once established, are less management and resource intensive than smaller-scale captive breeding colonies in zoos and wildlife parks. Given the species' small, overlapping home ranges of between 35 and 44 ha (Godsell 1983), large populations can be maintained within fenced reserves of modest size.

Insurance populations should be managed as a metapopulation, with individuals being transferred between captive populations to minimise deterioration of genetic variation for the species (Franklin and Frankham 1998). Care must also be taken in sourcing founder individuals from current low-density wild populations in Tasmania. While genetic diversity in insurance populations is desirable, it should not be at the expense of reducing wild populations to such low abundance as to render them unviable and functionally extinct, as occurred with wild source populations of eastern barred bandicoots in Victoria (Todd *et al.* 2002).

6.3.3.2 Mainland reintroductions

Reintroductions of eastern quolls into parts of their former distribution on the Australian mainland should also be considered. Plans are currently underway to reintroduce the species into fenced areas at Mulligans Flat (A. Manning, pers. comm.) and further reintroductions are being considered for North Head (J. Anson, pers. comm.), far-east Gippsland (A. Murray, pers. comm.), and NSW (T. Evans, pers. comm.). As previously noted for the insurance populations discussed at 6.3.3.1, care should be taken not to inadvertently reduce numbers or genetic diversity of wild populations in Tasmania should founder individuals be sourced from wild populations.

An understanding of population demographics and factors that affect eastern quoll population growth rates (such as differing population size, sex ratios and predator densities) should be considered in such reintroductions. Many reintroductions have failed as a result of too few founder individuals being introduced (Short *et al.* 1992; Christensen and Burrows 1995; Gibson *et al.* 1995; Pietsch 1995; Soderquist 1995), often because the species of interest is endangered and the availability of founder individuals is limited. To maximise the likelihood of success, it is important that the appropriate number of individuals is reintroduced to facilitate a net positive population increase. Sinclair *et al.* (1998) provided a list of important factors that should be considered prior to undertaking such reintroductions, including estimating the boundary density (the lowest density at which the reintroduced species and it's predators can coexist without a net reduction in population size), and whether per capita predation rates increase or decrease at low densities of the reintroduced species. The recommended study design at section 6.2.2 would estimate these key rates for eastern quolls, thereby enabling determination of the

minimum number of individuals to be reintroduced. However, should reintroductions occur before these rates are known, a precautionary approach should be adopted whereby the largest possible number of individuals is released, using a single large reintroduction in preference to multiple introductions of smaller numbers of individuals (McCallum *et al.* 1995; Sinclair *et al.* 2010).

6.4 Implications for global species conservation

The nature of the eastern quoll decline and the diagnosis of its potential causes are pertinent to conservation efforts globally. This case illustrates how quickly a common species can become rare, and shows the importance of appropriate monitoring programs to allow the timely identification and amelioration of declines. Effective management for recovery requires an understanding of the factors that limit a species' distribution and abundance. Ideally, these factors and how they interact should be understood before a species starts to decline, thereby allowing conservation managers to measure and determine what factors have changed to bring about the decline.

In prioritising conservation actions, common species such as the eastern quoll are often overlooked in favour of species that are unique, charismatic, naturally rare or imminently threatened with extinction. However, even a small proportional decline in abundance of a common species will result in the loss of a large number of individuals, with potentially far-reaching implications for ecosystem functioning (Ellison *et al.* 2005; Gaston and Fuller 2008; Gaston 2010; Lindenmayer *et al.* 2011). There are many examples of once common species being driven to extinction (Gaston and Fuller 2008). Frequently it is the common species that suffer most due to the effects of invasive alien species, as the large biomass and number of biotic interactions of common species will lead to pronounced cascades that impact a large number of other species (Gaston 2010).

This study also demonstrates how monitoring is essential for the early detection of a species decline, and for remedial action to commence on a timely basis, ideally before the capacity to restore is lost (Lindenmayer *et al.* 2011; Lindenmayer *et al.* 2012; Martin *et al.* 2012). For this reason, monitoring is particularly important for species conservation, even when the species is not currently threatened (Groom 2010). However large scale

monitoring programs can be expensive, and so limited conservation resources are often allocated away from common species in favour of monitoring and protecting species under higher extinction risk (Possingham *et al.* 2002; Field *et al.* 2005). Monitoring all species in all places is rarely practical; informed decisions need to be made about where monitoring resources are allocated based on ecological, economic and environmental grounds (Field *et al.* 2005; McDonald-Madden *et al.* 2010; Lindenmayer *et al.* 2012).

For a declining species threatened with extinction, monitoring can also provide important information on changes in key threats, such as changes in abundance or spatial distribution of a predator. Beyond this, monitoring is also a fundamental part of evaluating the success or failure of mitigation measures enacted to halt or reverse a species decline, thereby ensuring that management actions remain adaptive (Stem *et al.* 2005; Lindenmayer *et al.* 2012). But monitoring alone will not conserve species. Monitoring programs need to explicitly define critical trigger points for action, together with protocols for what actions are to be taken once those trigger points are reached (Lindenmayer *et al.* 2013). Otherwise, monitoring of declining populations will serve only to document extinctions (Martin *et al.* 2012).

This research further highlights the importance of understanding ecological interactions that influence the distribution and abundance of species, in particular the key threats and synergistic associations, ideally before a species declines. However there is a paucity of baseline information on the key threats for many species; often investigations only commence after the decline has become apparent. Indeed, this study has demonstrated how little was known about the threats to the eastern quoll before its decline, with most of the perceived threats based on anecdotal, localised or broad correlative observations (Jones 2000; Jones *et al.* 2003; Peacock and Abbott 2014) or risks inferred from related or similar sized species in Tasmania and on the mainland (Woinarski *et al.* 2014). However, the confounding of purported agents of decline often permits more than one plausible explanation, creating uncertainty about which factor(s) may be associated with the decline. While these observations may help to generate multiple hypotheses of causality, they do not provide evidence of cause and effect. For example, my research into the effects of *T. gondii* infection in eastern quolls demonstrated that while the high prevalence of *T. gondii* infection correlated with sites where quolls had declined, infection

did not affect quoll survival or reproduction, thereby discounting it as a causal agent in the decline. As we are unable to go back in time and measure how the candidate causal factors have changed, or the direction of any such change, valuable remediation time is lost in trying to ascertain the extent to which the perceived threats actually constitute real threats to the species.

An understanding of the causes of a species' decline is essential for the development and implementation of effective recovery plans. Understanding complex interactions may require complex modelling of systems to better appreciate the synergistic effects of multiple interacting factors. However field-based experiments, such as I have commenced in this study, will always be needed to understand the mechanisms and quantify the direction, variability and magnitude of effects so that models may be informative (Holt and Polis 1997; Linnell and Strand 2000). But we often are unable to defer management decisions until after appropriate experiments can be done to elucidate what these key threats may be and how they may interact. By that time, the species may have ceased to exist (Soulé 1985; McCallum 2000).

This study demonstrates how multiple threatening processes can interact, either sequentially or simultaneously, to bring about a species' decline and inhibit its recovery. Confounding variables and mechanisms can operate at different temporal and spatial scales. This is often the case for a species undergoing decline, where the final step in the extinction vortex may be unrelated or disconnected from the original cause of decline, and a suite of pervasive secondary processes and synergistic feedbacks eventually bring about the species' extinction. Small population size is not a cause of decline, but rather an effect of decline that can reinforce the actions of other causal agents, thereby increasing the species' risk of extinction. Detecting, diagnosing and halting species decline are some of the most challenging tasks faced by conservation practitioners. Current conservation practices focus predominantly on the remedial conservation and management of currently threatened species. However conservation biology will only become truly effective when practices anticipate and prevent future species' declines.

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Supplementary material

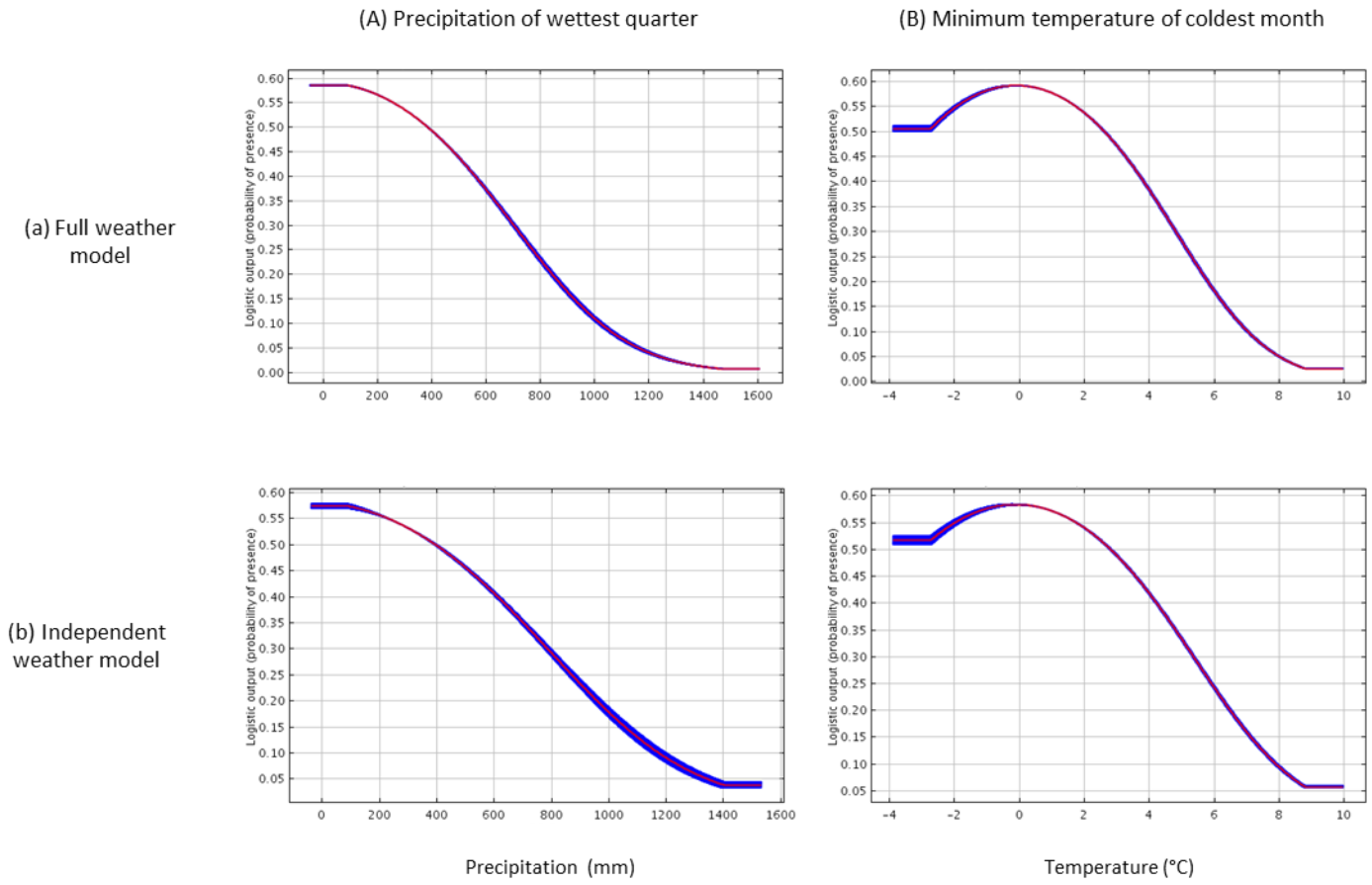

Figure S1. Response curves of eastern quolls (probability of presence) for the two most important weather variables: (A) precipitation of wettest quarter; and (B) minimum temperature of coldest month, in Tasmania. Response curves are shown for (a) full weather model (12 and 36 month variables) and (b) independent weather model excluding spotlight survey data. For all curves, the y axis indicates how predicted suitability (probability of presence) is dependent on (A) precipitation (mm) or (B) temperature (°C) shown on the x axis. The red curve shows mean response of 10 replicate runs used to cross-validate the model, blue shading indicates ± one standard deviation.

Table S1. Correlation matrices for the eight climatic variables used in weather models for the eastern quoll in Tasmania (1950-2009). (A) The correlations for each of the eight variables between the two time periods (12 months and 36 months). (B) The correlations between the eight variables used in the final weather model.

(A)		36 month
Annual mean temperature (bc01)	12 month	0.80
Temperature seasonality (bc04)	12 month	0.55
Max temperature warmest month (bc05)	12 month	0.56
Min temperature coldest month (bc06)	12 month	0.60
Annual precipitation (bc12)	12 month	0.62
Precipitation seasonality (bc15)	12 month	0.40
Precipitation wettest quarter (bc16)	12 month	0.57
Precipitation driest quarter (bc17)	12 month	0.55

(B)	bc01.12m	bc04.36m	bc05.36m	bc06.36m	bc12.36m	bc15.36m	bc16.36m
bc04.36m	0.28						
bc05.36m	0.49	0.70					
bc06.36m	0.47	(0.07)	0.14				
bc12.36m	(0.36)	0.00	(0.24)	(0.07)			
bc15.36m	0.01	0.03	0.25	0.13	(0.04)		
bc16.36m	(0.19)	0.03	(0.13)	0.16	0.82	0.39	
bc17.36m	(0.25)	(0.21)	(0.47)	(0.01)	0.58	(0.61)	0.22

Table S2. Estimated abundance of Tasmanian devils, feral cats and eastern quolls across 12 statewide camera sites. Estimates calculated using Royle Nichols model (Royle and Nichols 2003). Site names listed for each site code in Table 5.2.

	Tasmanian devils		Feral cats		Eastern quolls	
Site	Estimated abundance	95% confidence intervals	Estimated abundance	95% confidence intervals	Estimated abundance	95% confidence intervals
B	72	57-88	0	0-0	39	28-50
BL	168	144-192	441	401-481	4	2-7
BP	71	56-87	63	49-79	85	69-99
CFB	48	36-62	133	111-156	28	20-37
DE	167	143-191	94	76-114	7	4-11
FR	0	0-0	98	79-117	7	4-11
LE	28	19-38	64	49-80	4	2-7
LL	126	106-148	64	49-80	82	66-97
RO	132	111-154	171	146-196	0	0-0
SBI	0	0-0	172	147-198	0	0-0
UB	72	57-89	215	187-244	55	42-69
WNR	60	46-75	132	110-154	14	9-20

Video S1. Dynamic weather model, showing monthly variation in eastern quoll core habitat from 1950 to 2012. Note that video file has not been clipped to the Tasmanian coastline but instead presents every 5km x 5km grid cell containing a land surface, including islands. Accordingly, the outline appearance may differ slightly from the static maps provided at Figure 2.1 and Figure 2.3.

See GIF file on CD inside back cover.

Appendix A
Rapid decline in detections of the Tasmanian bettong (*Bettongia gaimardi*) following local incursion of feral cats (*Felis catus*).

Bettongia gaimardi detected in the February 2012 carnivore camera surveys at Judbury, Tasmania, prior to their local disappearance.

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A.1 Abstract

An abrupt decline in the number of Tasmanian bettongs (*Bettongia gaimardi*) was observed as part of a study investigating population declines in the eastern quoll (*Dasyurus viverrinus*). Seven remote camera surveys were undertaken at a monitoring site between February 2012 and October 2013. An 11% reduction in bettong detections was observed immediately following the first appearance of feral cats (*Felis catus*; at least three individuals) at the site. Within four months, bettong detections had fallen by 58% and by 100% within six months. No bettongs were detected in subsequent surveys undertaken 10, 12 and 16 months after cats were first observed. Cat predation and toxoplasmosis are discussed as mechanisms possibly responsible for the local disappearance of bettongs from this site, together with implications for the future management and conservation of the species.

A.2 Introduction

The Tasmanian bettong, or eastern bettong, is considered the most stable of the four species in the genus *Bettongia* (Rose and Johnson 2008). It is thought to have gone extinct on the mainland almost 100 years ago and is now found only in Tasmania (Wakefield 1967; Rose 1986; Menkhorst 2008; Rose and Johnson 2008). The demise of the species on the mainland has been attributed to predation by foxes (*Vulpes vulpes*) (Short 1998), however, persecution by humans and competitive grazing pressure from rabbits and livestock have also been implicated (Rose 1986; Maxwell *et al.* 1996; Menkhorst 2008).

In Tasmania, the species is still considered common and widespread (Maxwell *et al.* 1996; Menkhorst 2008; Rose and Johnson 2008). It is currently listed by the IUCN as near threatened (Menkhorst 2008) but is absent from federal and Tasmanian state listings of threatened species. Current threats in Tasmania include loss of habitat due to timber harvesting, excessive stock grazing and the use of 1080 poison for macropod control (Statham 1983; Rose 1986; Maxwell *et al.* 1996; Menkhorst 2008). Accordingly, current recommendations for the conservation and management of the Tasmanian bettong focus predominantly on the retention and management of suitable habitat and the controlled

use of 1080 poison in areas supporting vulnerable bettong populations (Rose 1986; Maxwell *et al.* 1996; Menkhorst 2008).

The recent introduction of foxes into Tasmania also presents an emerging threat for the Tasmanian bettong (Menkhorst 2008; Sarre *et al.* 2012). While foxes are considered a significant predation threat to the species (Short 1998), it is not known whether other eutherian carnivores such as feral cats depredate bettongs in Tasmania (Rose 1986). Feral cats are notably absent from the list of threats for the species (Maxwell *et al.* 1996; Menkhorst 2008), and there have been no investigations into the potential interactions between feral cats and Tasmanian bettongs.

As part of a study investigating population declines in the eastern quoll in Tasmania, remote camera surveys were used to monitor temporal changes in local mammal communities over a period of 21 months. This note reports an incidental observation of abrupt changes in the number of bettongs detected as part of these surveys, coinciding with the incursion of feral cats into the site.

A.3 Materials and methods

A series of longitudinal remote camera surveys was conducted on a cattle grazing property south of Judbury in southern Tasmania (43°01'24"S, 146°54'50"E). The property comprised large areas of open grazing pasture, adjacent to intact native eucalypt forest with minimal or no understorey.

Camera survey design

Seven camera surveys were undertaken during 2012 (February, June, October, December) and 2013 (April/May, June, October) and were optimised to investigate the spatial and temporal activity of eastern quolls. For each survey, 20 RECONYX™ PC800 passive infrared motion detector cameras were deployed for a minimum of 21 nights. Cameras were positioned approximately 50 m apart along a roughly linear 1 km transect that followed an interface between open pasture and eucalypt forest. The location and setup of cameras were standardised for all seven surveys, ensuring that any spatial bias in detection remained consistent across surveys. Each camera was fastened to a tree approximately 1.5 m above the ground, with a muttonbird oil scent lure positioned 2 to

3 m in front of each camera. For each trigger, cameras were programmed to take three pictures in rapid succession, with images taken continuously in further groups of three until all movement ceased. An infrared flash was used to illuminate images at night. All images were stamped with the time, date and camera number. While surveys were designed to target carnivore species, all mammal observations were recorded for each survey.

As most bettongs lack any distinguishing marks or features to facilitate identification of individual animals, activity was used as an index for the number of individuals detected. To minimise repeat captures of the same individual, a single detection or 'activity' was considered independent if it occurred >10 minutes after the last series of images for that species on that camera.

Additional survey data

In addition to the camera surveys, spotlight and trapping surveys were conducted along the camera transect. Vehicle-based spotlight surveys were undertaken twice every second month between September 2011 and July 2012, with five additional surveys in January, May and July 2013. Variables such as vehicle type, speed, observer, spotlight specifications, time and duration of survey were standardised across surveys. Each survey followed the same route along the bush-pasture interface and commenced around 60 minutes after dusk. All mammals observed during spotlight surveys were recorded, thereby providing additional survey data on bettongs and feral cats.

Carnivore trapping surveys were conducted every second month between May 2011 and July 2012, with additional surveys in January, May and July 2013. For each survey, 30 standard PVC mammal pipe traps were baited with lamb heart and set for three consecutive nights, spaced 30 to 50m apart along the same transect as the cameras. Only carnivores were captured in traps, providing additional capture data for feral cats but not for bettongs. To eliminate possible interference between survey methods, spotlight surveys were conducted in the week before and the week after trapping surveys. The second spotlight survey in July 2013 was not conducted due to localised flooding preventing access to the study site.

A.4 Results

Bettong activity

The number of bettong detections decreased across each of the first four camera surveys conducted between February and December 2012. Sixty two bettong detections were recorded in the first survey in February 2012. Activity decreased by 11% to 55 detections in June. By October, activity had fallen by 58% to only 26 detections. No bettongs were detected in the December 2012 survey or in any of the surveys conducted in April/May, June or October 2013 (Figure A.1). Spatial activity of bettongs was spread evenly along the transect in each camera survey. Of the 20 cameras deployed, bettongs were detected on 19 cameras in February, 18 cameras in June and 15 cameras in October 2012 and none thereafter.

Similar reductions were observed in spotlight surveys, although the reduction in early 2012 slightly preceded the decline in detections from camera surveys. The mean number of bettongs detected per survey reduced from 1.13 (range: 0.00-2.00) between September 2011 and March 2012, down to 0.25 (range: 0.00-1.00) between May and July 2012. No bettongs were detected in the five spotlight surveys conducted between January and July 2013 (Figure A.1).

All observed bettong activity occurred during nocturnal hours, although temporal activity patterns varied between seasons (Figure A.2). Surveys in February indicated a bimodal peak in activity. The first peak occurred during the first two hours after sunset, and a second larger peak occurred between 11 pm and 2 am (EST). Activity was lower at all other times of night but ceased by sunrise. Bettong activity in the June and October surveys followed a different pattern. Both surveys revealed a single peak in activity, with 62% of bettong activity concentrated in the three hours after sunset, and all activity ceasing two to three hours before sunrise.

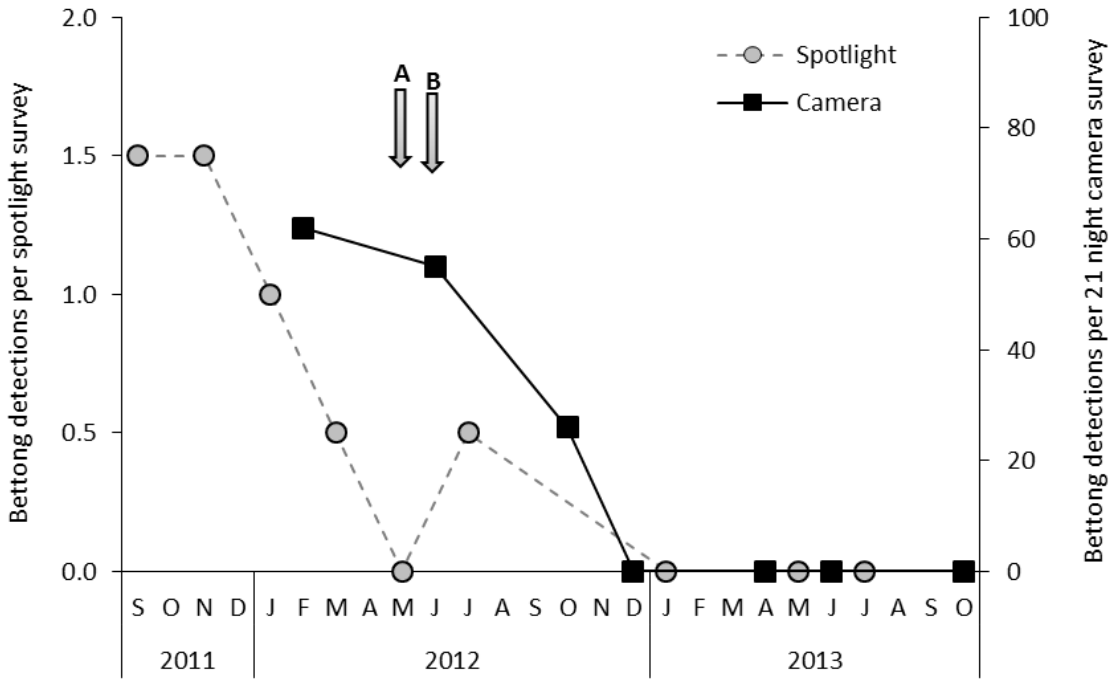

Figure A.1. Reduction in the number of bettong detections from spotlight and camera surveys conducted at Judbury between September 2011 and October 2013. Left axis shows the mean number of bettong detections per spotlight survey each period (grey circles) and the right axis shows the total number of bettong detections per camera survey (i.e. per 20 cameras) (black squares). Arrows indicate the point when feral cats were first detected both in trapping and spotlight surveys (A) and in camera surveys (B).

Feral cat activity

There was no evidence of feral cats in the eight spotlight surveys conducted between September 2011 and March 2012 nor in the six trapping surveys (540 trap nights) undertaken between May 2011 and March 2012. No feral cats were detected across 440 camera nights during the February 2012 camera survey (Figure A.2(a)).

A feral cat was first observed at the site during a spotlight survey undertaken on 16 May 2012. Two cats were subsequently trapped and removed on 21 May and a third cat on 22 May. Following removal of these cats, four more cat detections (between 2 and 4 individual cats) were recorded in camera surveys during June (Figure A.2(b)). A fourth cat was trapped and removed on 19 July. Four more cat detections (between 1 and 4 individuals) were recorded in camera surveys during October (Figure A.2(c)), but no cats were detected in the December camera survey. A fifth cat was trapped and removed on 17 May 2013. A single cat was detected in a spotlight survey conducted on 18 May, on camera on 4 May and again on 9 June. A sixth cat was trapped and removed on the last day of trapping on 21 July. The final camera survey recorded two cat detections (between 1 and 2 individuals) on 3 and 4 October 2013, indicating a minimum of seven individual cats was detected at this site between May 2012 and October 2013. Feral cat activity occurred across the entire length of the camera transect. In total, 75% (9/12) of cat detections occurred either in daylight hours or during the first 2 hours after sunset and the last 2 hours prior to sunrise, although temporal activity of cats varied between surveys. During the June and October 2012 camera surveys (when bettongs were declining), 88% (7/8) of cat detections occurred during the hours when bettongs were active (Figure A.2(b) and Figure A.2(c)).

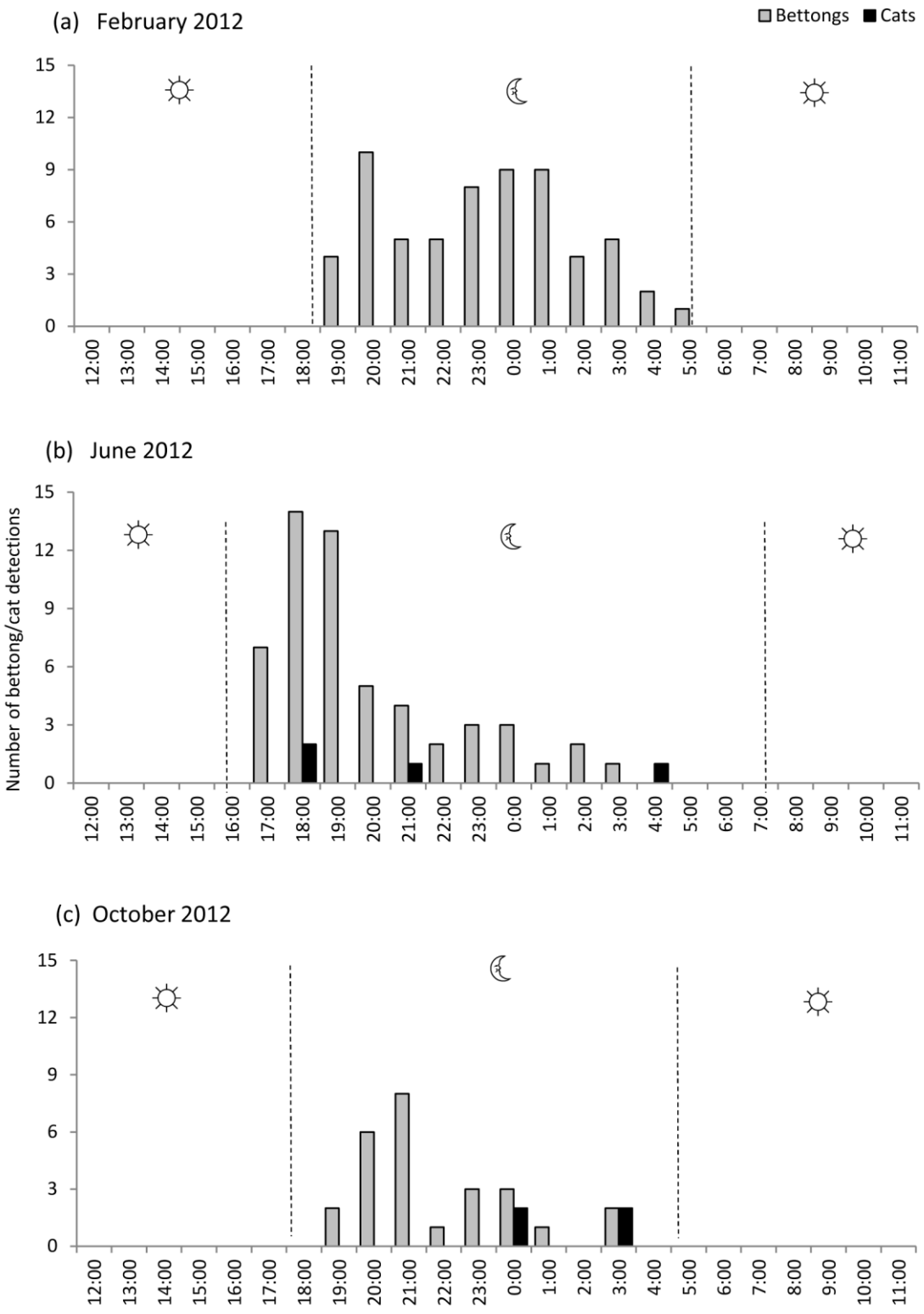

Figure A.2. Activity time of bettongs (grey bars) and feral cats (black bars) from camera surveys at Judbury, Tasmania. Results are shown for (a) February 2012 (*n* = 62 bettongs, 0 cats), (b) June 2012 (*n* = 55 bettongs, 4 cats) and (c) October 2012 (*n* = 26 bettongs, 4 cats). Vertical axis show the total number of bettong or cat detections in each hourly time block across all 20 cameras over 21 nights. Vertical broken lines indicate sunset and sunrise times for each survey. All times presented using Australian Eastern Standard Time (AEST).

A.5 Discussion

An abrupt decline in bettong detection rates was observed in the current study, coinciding with the appearance of feral cats at the site for the first time. Within seven months of cat incursion, bettongs had disappeared from the site and were not detected during a further 12 months of monitoring. Further investigations are required to first confirm whether the observed decline in activity represents a real decline in bettong abundance, ideally by corroborating capture-mark-recapture data results with a range of alternative survey methods (e.g. Fancourt *et al.* 2013; Wayne *et al.* 2013). While the relationship between activity and abundance has not been specifically assessed here, camera detections have yielded similar abundance estimates to alternative survey methods such as live trapping and line-transects for a range of species (Trolle *et al.* 2008; De Bondi *et al.* 2010), including eastern quolls and Tasmanian devils (*Sarcophilus harrisii*) at this study site (B. Fancourt, unpubl. data). This suggests that the observed decline in bettong activity may reasonably signal a decline in abundance.

Detection of bettongs

The use of camera lures to specifically target carnivores may have resulted in unreliable estimates for non-carnivore species such as bettongs. However, few images revealed bettongs investigating the carnivore lures, with most images capturing bettongs incidentally as they moved past the camera. Accordingly, the carnivore lure was probably not a major attractant for bettongs, and any reduction in lure effectiveness across surveys is unlikely to explain the observed decline in bettong detections. Moreover, spotlight surveys revealed similar rates of decline, although the decline in spotlight observations appears to have preceded that observed in the camera surveys. Analysis of activity times from camera surveys revealed that nightly bettong activity was greatest around the time that spotlight surveys were conducted, suggesting the temporal difference is not an artefact of seasonal variation in activity. The difference may simply reflect the inherent weakness of spotlight survey data, with a brief snapshot on a single night likely to miss or underestimate activity that will more easily be detected by remote cameras left *in situ* for three continuous weeks. Notwithstanding these slight temporal differences, both camera and spotlight surveys frequently detected bettongs up to November 2012, and both

methods failed to detect any bettongs in the 11 months thereafter, suggesting that the observed decline and subsequent disappearance is real.

Detection of feral cats

The first observation of feral cats in May 2012 suggests the time of feral cat incursion at this site. As the incursion could not have been foreseen, only one camera survey was conducted prior to the appearance of cats. Accordingly, it is not possible to ascertain whether feral cats were present but not detected in the first camera survey in February 2012, or if cats were totally absent prior to their first detection in May 2012. However, as all three survey methods failed to detect any cats prior to May 2012 (traps and spotlight) or June 2012 (cameras), the presence of cats at this site appears highly unlikely in the 12 months prior to their first detection in May 2012. Even as six cats were progressively trapped and removed from the site throughout the study, cats continued to be trapped up to the last day of trapping surveys in July 2013 and detected up to the last camera survey in October 2013, indicating that the combination of complimentary survey methods used in the current study was adequate to detect the presence of cats at this site.

Causes of the decline

While observations suggest that feral cats may be the agent of bettong decline at this site, this evidence is entirely correlative and therefore does not demonstrate causation. Further investigations are required to identify whether feral cats are responsible for the rapid disappearance of bettongs from this site, and if so, by what mechanism. I discuss three potential hypotheses that warrant further testing.

1) Predation

Predation by feral cats could explain the rapid decline in bettongs. While at least seven individual cats were detected across a period of 18 months, no more than four were detected on the site at any time, with cats captured in traps being removed and euthanased upon first capture. However, numerous studies have demonstrated that predation by a single cat can be catastrophic to vulnerable wildlife populations in short periods. For example, Gibson *et al.* (1994) confirmed that feral cats were responsible for the death of 40% of reintroduced rufous hare-wallabies (*Lagorchestes hirsutus*) released

into the southern Tanami Desert, and a further 56% of hare-wallabies released into the western Tanami Desert. While feral cats were present in both release areas before, during and after release, many hare-wallabies survived for extended periods before they were suddenly killed within a 2 to 4 week period. A single cat at each site was believed to be responsible for the predation. Once these two cats were killed, no further predation occurred during the next 2 to 3 years, despite continued trapping and track monitoring indicating that cats were still present.

While it is unknown whether feral cats prey on Tasmanian bettongs (Rose 1986), they are known to be a significant predator of other bettong species including the critically endangered brush-tailed bettong or 'woylie' (*Bettongia penicillata*) and the burrowing bettong or 'boodie' (*Bettongia lesueur*). For example, feral cats were identified as the main cause of mortality of reintroduced woylie populations in New South Wales (Priddel and Wheeler 2004) and South Australia (SA) (Copley *et al.* 1999; James *et al.* 2002) and of many individuals in indigenous populations in Western Australia (WA) (Marlow and Williams 2012). Christensen and Burrows (1995) determined that feral cats were also responsible for the rapid and complete demise of translocated burrowing bettongs released into the Gibson Desert Nature Reserve in WA.

The Tasmanian bettong is only slightly larger than both *B. penicillata* and *B. lesueur*, but is similar in size to *L. hirsutus*, suggesting that predation by feral cats is entirely possible. Analysis of activity times confirms that feral cat activity was concentrated into the hours when bettongs were also active, thereby presenting bettongs with a high likelihood of encountering a cat. To ascertain whether bettongs were killed by cats in the current study, bettong carcasses would need to have been recovered on a timely basis to allow post-mortem investigation and ideally diagnose cause of death. However, as the disappearance of bettongs was observed incidentally while analysing camera data for carnivore species, bettong carcasses were not recovered.

2) Exclusion

The incursion of feral cats may have forced bettongs away from the study site into safer areas beyond those monitored in the current study. As the home range of a bettong is around 61 ha (Taylor 1993) and cameras in the current study covered only a 1 km linear

transect, it is possible that bettongs may have left the study area in the months following cat arrival. While habitat shift to avoid predation has been observed in studies of both terrestrial and aquatic prey species (Sih 1984), there is no evidence from studies on this or related species indicating that this is likely. The limited static area covered by the cameras in the current study precluded any investigation of whether declines at the study site were offset by an equivalent increase in surrounding areas. Surveys extending over larger areas beyond the bettong's usual home range would be required to determine whether localised declines represent spatial shifts over time or true population declines.

3) Toxoplasmosis

Feral cats may have exposed bettongs to the disease toxoplasmosis. Cats are the definitive host of *Toxoplasma gondii*, a coccidian parasite that causes the disease toxoplasmosis (Frenkel *et al.* 1970). All mammal and bird species can acquire the parasite, often through the consumption of food, soil or water contaminated with infective oocysts that are shed by cats in their faeces (Dubey *et al.* 1970b; Frenkel *et al.* 1970; Miller *et al.* 1972). While infection is typically subclinical in most individuals (Dubey *et al.* 1988; Hill and Dubey 2002), pathogenicity and clinical signs vary between species and individuals (Attwood *et al.* 1975; Obendorf and Munday 1983; 1990; Burns *et al.* 2003). Toxoplasmosis may not always result in direct mortality, however the effects of overt clinical disease may increase the likelihood of predation on free-ranging infected hosts.

Australian marsupials in general are susceptible to toxoplasmosis (Obendorf and Munday 1983; Canfield *et al.* 1990; Innes 1997; Bettiol *et al.* 2000), but the susceptibility of Tasmanian bettongs is unknown. As part of a pre-translocation health evaluation of free-ranging Tasmanian bettongs in 2011-12, Portas *et al.* (2014) found the seroprevalence of *T. gondii*-specific IgG antibodies was 0% (0/59). *Prima facie* this may suggest a low exposure of bettongs to *T. gondii*. However, the Tasmanian mainland has an exceptionally high prevalence of *T. gondii* across the state (Fancourt and Jackson 2014 [Chapter 4]). Furthermore, the foraging ecology of bettongs predisposes them to a high risk of exposure to infective oocysts as they dig for hypogeous ectomycorrhizal fungi in soil where feral cats defaecate (Johnson 1994), suggesting a high risk of exposure to *T. gondii*. Alternatively, bettongs may be highly susceptible to clinical toxoplasmosis, with affected individuals being rapidly removed from the population and only unexposed individuals

remaining to be sampled. This would result in an observed low seroprevalence in free-ranging populations (McCallum 1994) and may explain the absence of any seropositive bettongs by Portas *et al.* (2014). A low seroprevalence has also been observed in populations of the rapidly declining brush-tailed bettong in WA and SA (Parameswaran *et al.* 2008). While the prevalence of *T. gondii* has been associated with declining brush-tailed bettong populations, the significance of the association as a causative agent of decline is still under investigation (Wayne *et al.* 2011).

While Tasmanian bettongs were not tested for seroprevalence of *T. gondii*-specific IgG antibodies in the current study, *T. gondii* was highly prevalent at the study site. All feral cats trapped at the site (6/6) were seropositive (Fancourt and Jackson 2014 [Chapter 4]). Furthermore, 85% (29/34) of eastern quolls tested at this site between May 2011 and July 2013 were seropositive (Fancourt *et al.* 2014 [Chapter 3]). This confirms a high level of *T. gondii* contamination and a significant risk of exposure for bettongs at this site. Further research is recommended to determine whether Tasmanian bettongs are susceptible to clinical toxoplasmosis, either through direct mortality or through an increased risk of predation for infected individuals.

Implications and future research

Further studies are required to establish whether the observed decline in bettong detections is indicative of a real decline, and to investigate the responsible mechanism(s). The observations in the current study suggest that feral cats may pose a significant threat to Tasmanian bettongs, with important implications for the future conservation and management of the species.

While cats have been in Tasmania for over 200 years (Abbott 2002), the decline of the Tasmanian devil with the spread of the fatal Devil Facial Tumour Disease (Hawkins *et al.* 2006) may release feral cats from competitive pressure, possibly resulting in spatial and temporal shifts in hunting activity and perhaps an increase in abundance. Such changes would present an increased risk of predation and toxoplasmosis to susceptible species. Accordingly, it is crucial that interactions between feral cats and bettongs be investigated to enable adaptive management of potentially vulnerable bettong populations.

The observations in the current study are also pertinent to current reintroductions of Tasmanian bettongs into parts of their former range on the Australian mainland (Shorthouse *et al.* 2012). A number of free-ranging bettongs have been translocated into predator-proof enclosures in the Australian Capital Territory, with future plans to release bettongs into areas beyond the fenced enclosures (A. Manning, pers. comm.). While planned release sites will be baited to control local fox populations (A. Manning, pers. comm.), some fox baiting programs in WA have observed an increase in feral cats following the removal of foxes (Christensen and Burrows 1995; de Tores 2012). While further research is urgently required to understand if feral cats are indeed a threat to the Tasmanian bettong, a precautionary approach should be adopted in the interim management of the species. Feral cat control or exclusion from vulnerable bettong populations should be considered an essential component of adaptive management actions to ensure conservation of the species both in Tasmania and in mainland reintroduction programs.